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Original articles

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Original article

The prophylaxis of venous thromboembolism in medical outpatients: results of a survey among italian general practitioners

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Summary. *Background:* Although the majority of venous thromboembolic events occurs in primary care, most of the studies concerning its prophylaxis investigate hospitalized patients. Therefore, in primary care, many clinical decisions have to be taken in the absence of great clinical evidence derived from studies performed directly on outpatients. The objective of our study is to evaluate the clinical approach of Italian General Practitioners to the prophylaxis of venous thromboembolism in medical outpatients. *Methods:* A web-based questionnaire was emailed to 766 Italian General Practitioners. In the questionnaire there were four exemplary clinical cases concerning hypothetical patients at venous thromboembolic risk. *Results:* Overall 232 questionnaires were returned. Approximately 40% of the participants reported to assess thrombotic and hemorrhagic risk with a risk assessment model but nevertheless only a narrow minority had recourse to a suitable and validated score for this purpose. In the chronically bedridden patient about half of the participants administered a heparin or an antiplatelet drug for long time. In acute outpatients at high venous thromboembolic risk there was a considerable underuse of heparin prophylaxis and graduated compression stockings were often considered as a first prophylactic option. Prolonged heparin prophylaxis in the post-acute setting was also the practice for half of the participants. *Conclusions:* Italian General Practitioners approach these "grey" areas of uncertainty in a significantly heterogeneous way and sometimes in sharp contrast to the recent evidence. The present findings stress the need for further targeted educational programs and new high quality studies to further deep this clinical context. (www.actabiomedica.it)

Key words: bedridden persons, family practice, outpatients, risk assessment, venous thromboembolism

Introduction

Venous thromboembolism (VTE) is one of the most important public health problems, due to its high incidence and morbidity, which has a significant impact in terms of consumption of health resources (1, 2). Antithrombotic prophylaxis may be a useful strategy to contain the problem. Despite this, thromboprophylaxis remains largely underused in many different clinical settings (3-6).

While the majority of VTE events occurs in primary care (7), almost all of the studies concerning its prophylaxis investigate hospitalized patients. Furthermore, risk assessment models (RAMs) for VTE have been validated, till now date, only for hospitalized patients. Therefore, in primary care, many clinical deci-

sions have to be taken in the absence of great clinical evidence derived from studies performed directly on outpatients.

For example, very few studies have evaluated the efficacy and safety of VTE prophylaxis both from a pharmacological and a mechanical point of view, in home-assisted non-surgical patients with acute medical problems. Despite a general perception occurrence of VTE out of hospital appears similar to in hospital both for risk factors and prognosis (8, 9). The aim of our study is therefore to evaluate the clinical approach of Italian General Practitioners (GPs) to the prophylaxis of VTE in medical outpatients. We conducted a survey among a large cohort of GPs to measure their decision orientation in some important "grey" areas of VTE prevention in the context of primary care.

Methods

Design and questionnaire

A web-based questionnaire was emailed to all 766 GPs of Local Health Authorities of Central-South Piedmont, a region in northwest Italy. Data collection was conducted from April 2018 to June 2018. All individual email addresses were obtained from the databases of Local Health Authorities of Central-South Piedmont. Emails contained a general description of the survey and an invitation to participate through a web-based link. A pilot version of the questionnaire was previously sent to 10 external GPs. They were interviewed after filling out the pilot version in order to check the correct functioning of web-based system and to assure the clarity of questions.

The definitive questionnaire consisted of a first part in which the participant's general information was collected, such as: gender, age, years of activity as GP, participation in at least a conference concerning the VTE over the last five years, assessment of thrombotic and hemorrhagic risk of a patient (whether clinically or through a RAM).

In the second part of the questionnaire, there were four exemplary clinical cases concerning hypothetical patients at VTE risk. For each of the four scenarios, three or four alternatives of choice were proposed regarding the possible optimal antithrombotic prophylaxis (Table 1).

In short, the first scenario described a patient suffering from Parkinson's disease and chronically bedridden. The second case was a cancer patient with a severe renal insufficiency and an acute urinary tract infection. The third scenario analyzed the situation of a patient with an acute heart failure relapse with respiratory failure treated at home. Finally, the last clinical case described a diabetic patient, previously hospitalized for an exacerbation of chronic obstructive pulmonary disease (COPD), discharged to his home to continue antibiotic therapy.

Statistical analysis

All the obtained answers were inserted into an anonymous database and subsequently analyzed. Continuous variables were expressed as mean and standard deviation (SD); categorical data and qualitative variables instead as counts and percentages. Subgroup analyses were performed including only physicians with clinical experience longer than ten years and doctors who have attended at least one conference concerning VTE in the last five years. Statistical analyzes were performed using the SPSS program version 23.0.

Results

Overall 232 questionnaires were returned (30.3% of the whole sample). Baseline characteristics of responders are summarized in Table 2; 130 GPs (56.0%) were male. Responders' mean age was 52.4±13.1 years with an average service length of 21.5±14.6 years; 136 GPs (58.6%) attended at least one conference regarding the topic of VTE n the last 5 years; 96 participants (41.4%) claimed to use a specific RAM for thromboembolic risk. The scores used were: CHA2DS2-VASC (42 participants, 18.1% of the total sample), PADUA (20 participants, 8.6%), WELLS (19 participants, 8.2%), CAPRINI (2 participants, 0.9%) and GENEVA (1 participant, 0.4%).

The risk of hemorrhagic complications was assessed through the use of a RAM by 95 participants (40.1%). The scores adopted were: HASBLEED (78

Table 1. The four exemplary clinical cases

Case 1
91-years-old woman
Past medical history: Parkinson's disease;
History of the present illness: In the last year the patient has gradually lost autonomy in the activities of daily life and at the present time is chronically bedridden.
Which of the following prophylactic therapies do you consider appropriate?
1. LMWH at prophylactic dosage for long-term;
2. The patient does not need VTE prophylaxis;
3. Antiplatelet drug (e.g. acetylsalicylic acid 100 mg/day);
4. Oral anticoagulant therapy with VKA.

Case 2
66-years-old man
Past medical history: Prostatic carcinoma with bone metastases treated with hormonal therapy, chronic renal failure IV stage (CrCl = 28 ml/min);
History of the present illness: For one day the patient has a high fever (>38°C) with shiver accompanied by dysuria and pyuria. Antibiotic therapy is started In the strong suspicion of infection of the lower urinary tract. The patient moves independently at home.
Which of the following prophylactic therapies do you consider appropriate?
1. The patient does not need VTE prophylaxis;
2. LMWH at prophylactic dosages for 10±4 days;
3. Prophylactic doses of UFH;
4. Antiplatelet drug (e.g. acetylsalicylic acid 100 mg/day).

Case 3
82-year-old woman
Past medical history: Heart failure with reduced ejection fraction (NYHA class II), previous transient ischemic attacks, polymyalgia rheumatica, moderate obesity, previous deep venous thrombosis (2 years ago);
History of the present illness: for about 3 days increase in peripheral edema with worsening of dyspnea (NYHA class III). In agreement with the family, heart failure relapse is treated at home. Intravenous diuretic therapy is initiated and oxygen supplementation by nasal cannula too. The patient is not currently bedridden and he retains autonomy in the activities of daily living.
Which of the following prophylactic therapies do you consider appropriate?
1. The patient does not need prophylaxis;
2. LMWH at prophylactic dosages;
3. Graduated compression stockings.

Case 4
77-year-old man
Past medical history: Type 2 diabetes mellitus, essential arterial hypertension, COPD stage 3C;
History of the present illness: The patient was hospitalized for 8 days in the department of internal medicine for bronchitic exacerbation. It has been treated with intravenous antibiotics, oxygen supplementation by nasal cannula and prophylactic-dose of LMWH. He was discharged with indication to continue oral antibiotic therapy for another 3 days and provided with home oxygen for the persistence of mild respiratory failure. The patient performs bed-chair passages and goes to the bathroom with the help of a caregiver.
Which of the following prophylactic therapies do you consider appropriate?
1. LMWH at prophylactic dosage until the complete resumption of walking or in any case for a period not exceeding 35 days;
2. LMWH at prophylactic dosage up to the 14th day from the beginning of the hospital;
3. The patient does not need VTE prophylaxis;
4. Antiplatelet drug (e.g. acetylsalicylic acid 100 mg/day).

ASA=acetylsalicylic acid, COPD=chronic obstructive pulmonary disease, CrCl=clearance of creatinine, LMWH=low-molecular-weight heparin, n=number, NYHA class=New York Heart Association functional classification of heart failure, UFH=unfractionated heparin, VKA=vitamin K antagonists, VTE=venous thromboembolism

Table 2. Baseline characteristics of responders

Male gender, n (%)	130 (56,0)
Mean age, years, m±ds	52.4 ± 13.1
Mean length of service, years, m±ds	21.5 ± 14.6
Attendance at least one conference concerning VTE in the last 5 years, n (%)	136 (58.6)
Thromboembolic risk evaluation with a RAM, n (%)	96 (41.4)
CHA2DS2–VASC score, n (%)	42 (18.1)
PADUA score, n (%)	20 (8.6)
WELLS score, n (%)	19 (8.2)
Not specified, n (%)	12 (5.2)
CAPRINI score, n (%)	2 (0.9)
GENEVA score, n (%)	1 (0.4)
Bleeding risk evaluation with a RAM, n (%)	95 (40.1)
HASBLEED score, n (%)	78 (33,6)
Not specified, n (%)	12 (5,2)
IMPROVE score, n (%)	4 (1,7)
HEMORR2HAGES score, n (%)	1 (0,4)

ds=deviation standard, m=mean, n=number, RAM=risk assessment model, VTE=venous thromboembolism

participants, 33.6% of the total sample), IMPROVE (4 participants, 1.7%) and HEMORR2HAGES (1 participant, 0.4%).

Result of four clinical scenarios are summarized in Table 3 and Table 4. In the first scenario almost half of the participants (47.0%) abstained from prescribing any prophylactic therapy, while about one third (29.3%) would have added an antiplatelet drug in therapy; 19.0% of responders adopted a low-molecular-weight heparin (LMWH) at prophylactic dose and 4.7% an oral anticoagulant therapy with a vitamin K antagonists (VKA).

In the second case, the majority of GPs (71.6%) did not prescribe any prophylactic therapy, whereas only 18.5% adopted a prophylactic dose of LMWH. The percentages of those who administered a prophylactic dose of unfractionated heparin (UFH) (6.0%) or of an antiplatelet drug (3.9%) were low.

In the third scenario, participants almost equally choose to abstain from any therapy (34.5%) or to use a prophylactic dose of LMWH (32.3%) or a graduated compression stockings (GCS) (33.2%).

In the last clinical case, the majority of responders (59.1%) preferred to continue a prophylactic dose of LMWH at until complete resumption of walking or for a period not exceeding 35 days; 19.0% prescribed LMWH prophylaxis for a period of 14 day. The percentages of those who did not prescribe any prophylaxis (14.6%) or administer an anti-aggregation (7.3%) were low.

Table 3. Results of first and second clinical scenarios and analysis of the subgroups

	case 1				case 2			
	1	2	3	4	1	2	3	4
All (232), n (%)	44 (19,0)	109 (4,0)	68 (29,3)	11 (4,7)	166 (71,6)	43 (18,5)	14 (6,0)	9 (3,9)
Clinical experience > 10 years (144), n (%)	25 (17,4)	60 (41,6)	52 (36,1)	7 (4,9)	101 (70,1)	28 (19,4)	6 (4,2)	9 (6,3)
Attendance at one conference concerning VTE in the last 5 years (136), n (%)	28 (20,6)	61 (44,9)	41 (30,1)	6 (4,4)	93 (68,4)	29 (21,4)	7 (5,1)	7 (5,1)
	LMWH	nothing	ASA	VKA	nothing	LMWH	UFH	ASA

ASA=acetylsalicylic acid, LMWH=low-molecular-weight heparin, n=number, UFH=unfractionated heparin, VKA=vitamin K antagonists, VTE=venous thromboembolism

Table 4. Results of third and fourth clinical scenarios and analysis of the subgroups

	case 3			case 4			
	1	2	3	1	2	3	4
All (232), n (%)	80 (34,5)	75 (32,3)	77 (33,2)	137 (59,1)	44 (19,0)	34 (14,6)	17 (7,3)
Clinical experience > 10 years (144), n (%)	46 (31,9)	57 (39,6)	41 (28,5)	85 (59,0)	34 (23,6)	12 (8,4)	13 (9,0)
Attendance at one conference concerning VTE in the last 5 years (136), n (%)	42 (30,8)	47 (34,6)	47 (34,6)	80 (58,9)	32 (23,5)	13 (9,5)	11 (8,1)
	nothing	LMWH	GCS	LMWH for 35 d	LMWH for 14 d	nothing	ASA

ASA=acetylsalicylic acid, d=days, GCS=graduated compression stockings, LMWH=low-molecular-weight heparin, n=number, UFH=unfractionated heparin, VKA=vitamin K antagonists, VTE=venous thromboembolism

The analysis of the subgroups comprising only participants with clinical experience over ten years and those who attended at least one conference concerning VTE over the last five years, showed similar and overlapping results to those observed in the main analysis.

Discussion

The results of this survey conducted among Italian GPs reflect a substantial heterogeneity in the clinical management of medical outpatients at risk of VTE. About 40% of participating GPs reported that they assess the risk of VTE and bleeding in their patients with the use of a RAM. However, the vast majority reported to use score developed and validated in other clinical settings and less than 10% of physicians reported to use the Padua Prediction Score (10) and the IMPROVE Bleeding Score (11), to evaluate the thrombotic and hemorrhagic risk as suggested by the most recent guidelines from the American College of Chest Physicians (ACCP) (12).

There is an open debate on whether to consider chronically bedridden patients at high risk of VTE (first scenario). Clinical data indicate that prolonged immobility represents a risk factor for VTE in the first thirty days of immobility and then its weight in terms of risk is reduced in the absence of intercurrent risk factors such as acute non-surgical disease (such as sepsis, exacerbated COPD, heart failure relapse, stroke). Therefore, in the absence of overlapping intercurrent risk factors, prolonged immobility beyond thirty days should not be considered a risk factor for VTE (13). This is in accordance with the latest guidelines of the ACCP that in chronically bedridden patients residing at their home or in a nursing home, recommended against the routine use of thromboprophylaxis (12). Nevertheless, in our study only less than half of the sample adopted this clinical behavior, and about one out of five GPs prescribed a prophylaxis with LMWH and almost one out of three GPs prescribed an antiplatelet agents which are not recommended for antithrombotic prophylaxis in medical patients due to their limited efficacy in this setting (14-16).

Evidence on the appropriate antithrombotic prophylaxis in patients with severe renal failure is lacking and evidence on the risk of bleeding associated with the use of these drugs in this setting is not compelling (12). Pharmaceutical company recommends a reduced daily dose of enoxaparin for patients with a clearance of creatinine (CrCl) less than 30 ml/min. Anti-factor Xa levels appeared slightly increased in a small cohort of patients with renal failure treated with prophylactic dose of this drug (17). Data on other LMWH are even more limited and it is not clear if the use of UFH is associated with a better efficacy and safety tradeoff in comparison to LMWH. Answer to the second scenario seem to be driven by the fear of bleeding complication and use of a prophylactic dose of heparin was suggested by approximately 25% of GP, with only a minority of participants choosing UFH.

In the international literature there is not convincing scientific evidence on the effectiveness of the use of mechanical devices in the prophylaxis of VTE in medical patients (18-20). The latest guidelines of the ACCP underline how mechanical devices are therefore an alternative for the prevention of VTE in medical patients at high risk of bleeding in which pharmacological prophylaxis is contraindicated or the benefit is not clear (12). Nevertheless the use of GCS in the third clinical case was considered by almost one third of the participants. In this circumstance, furthermore, they could promote venous return damaging the cardiac preload and aggravating heart failure disease.

Although we did not collect information about the logical reasoning of the responses, we can hypothesize that acute ill patients in third scenario were perceived to be at low risk of thromboembolic complications, also thanks to his apparently preserved mobility. However, it must be emphasized that the concept of reduced mobility does not exclusively define the patients confined to bed or armchair for the whole day but it must also be extended to those who perform, autonomously or with help, only modest movements from a room to another one at home (21). Furthermore, as underlined by the PADUA score, the risk of VTE is generally increased also during an acute cardiac (22), respiratory (23), infectious (24) and rheumatic (25) disease or for the presence of a thrombophilia (26) or an active cancer disease (27, 28).

The duration of pharmacological prophylaxis of VTE in the medical patient (fourth clinical case) remains uncertain (29). Despite the lack of clear evidences to support this strategy, most GPs considered the possibility of extending anticoagulant prophylaxis for a significant period beyond hospitalization in patients who potentially remain at higher risk for VTE. This would appear to be in contrast with the latest guidelines of the ACCP, where prolonged routine prophylaxis is discouraged beyond the period of immobilization or acute hospitalization of the patient (12). These recommendations are based on the negative results of three large RCTs, who compared respectively enoxaparin (30) and the direct oral inhibitors apixaban (31) and rivaroxaban (32) with placebo after an initial period of prophylaxis of up to 14 days. In all these studies, the potential benefit of prolonged prophylaxis was compromised by an increased risk of major bleeding complications.

The results of our survey suggest that Italian GPs approach the prophylaxis of VTE in medical outpatients in a heterogeneous way and sometimes deeply in contrast to current international recommendations. This would seem to follow the information obtained from previous similar studies concerning the clinical behaviors of Italian Internist Physicians (33). Both the poor familiarity with the published guidelines and the lack of clear evidence from studies specifically oriented to the world of primary care could explain these behaviors in clinical practice. Unfortunately, as previously pointed out, the structure of this survey don't allow, however, to gather various motivations and explanations regarding the different answers. Nevertheless, these results allow us to evaluate the current knowledge of a large group of GPs on the topic of VTE prophylaxis and provide us useful information on their attitude in different medical scenarios.

It is also interesting to note that almost 60% of GPs declared they have participated in at least one conference concerning the VTE over the last 5 years. However, the specific analysis of the data of this subgroup didn't show significant variability in comparison to the total sample questioning its effective impact on clinical practice. The information obtained from our survey could therefore be used as a starting point to plan future more targeted educational programs and new quality studies aimed at further deepening this clinical context.

Our study has some limitations. The response rate we have observed (30.3%) seems to be modest, but it is similar to results of most surveys performed among GPs. Actually, GPs' response rates to surveys are lower than those of the general population and often lower than 30% (34, 35). Moreover, response rates to web and email surveys are known to be lower than those of postal surveys (36). Most studies have found time and workload pressure, negative attitudes toward research, concerns about the researchers' motivations and lack of interest in the research as the main self-reported reasons for low participation (37, 38). Furthermore, our survey includes only Italian GPs and therefore the extrapolation to different foreign health systems can be questionable. In addition, physicians may have misin-

terpreted the questions and we cannot be sure that the participants gave reproductive answers to their clinical behavior in daily practice. To avoid these potential biases we have tried to structure the survey in the simplest and least equivocal form possible and collected the answers in a totally anonymous way.

Conclusion

In conclusion, the results of our survey provide real data on the current clinical management of antithrombotic prophylaxis in the context of Primary Care. Italian GPs approach these "grey" areas of uncertainty in a significantly heterogeneous way and sometimes in sharp contrast to the recent evidence of international scientific literature. All this reinforces the need for further targeted educational programs and new high quality studies to further deep this clinical context.

Conflict of interest: None to declare

Authors' contributions: All the authors participated in the conception and realization of the study. All the authors contributed in data interpretation and drafted the article and approved the final version to be published.

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Original article

Effects of D-Mannose, Ellirose™ and Lactobacillus Plantarum in treatment of urinary tract recurrent infections (rUTIs): A survey of urologists knowledge about its clinical application

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Summary. *Background and aim of the work:* Urinary tract infections (UTIs) and recurrent urinary tract infections (rUTIs) are widespread disease and almost half of all women will experience at least one episode of cystitis during their life. Aim of this study was to review the evidence of literature about the therapeutic and preventive effects of a product containing D-Mannose, Ellirose™ and Lactobacollus Plantarum on patients' symptoms, quality of life and recurrence of UTIs and to investigate the practicing urologists' knowledge about the clinical application of this product. *Materials:* We administrated an investigational survey about clinical use of a phytotherapeutic product made of D-Mannose, Ellirose™ and Lactobacollus Plantarum to 12 residents in Urology at the University of Modena and Reggio Emilia and to 32 urologists working in the provinces of Modena, Reggio Emilia and Parma. *Results:* 61% of physicians have diagnosed rUTIs in 3-6 patients during a month, and 7% of them in more than 6 patients during the same period of time. By these results rUTIs appear as common pathological conditions. 59% prescribed the product at least 1 time a month and 14% prescribed it more than 5 times. 43% administrated the product after out-patient invasive examinations as cistoscopy and urodynamic exam for UTIs prevention. 55% noticed a significant improvement in patient's QoL (Quality of Life) suffering from rUTIs. Furthermore 48% also reported a significant effect for the improvement of urinary symptoms of patients. No gastric or general side effects have been noticed during the administration period. Finally the cost of integrator has been reported affordable for the great majority of patients. *Conclusions:* Many studies in Literature have shown that D-Mannose and H. sabdariffa (Ellirose™) reduce the risk of development of rUTIs opposing colonization and proliferation of uropathogenic bacteria in urinary tract. Our investigational survey about the administration of a phytotherapeutic product showed that this product is well-known and has a proved positive impact. (www.actabiomedica.it)

Key words: urinary tract recurrent infections (rUTIs), investigational survey, D-Mannose, Ellirose™ and Lactobacillus Plantarum

Background and aim of the work

Urinary tract infections (UTIs) consist in the presence of microbial pathogens in the urinary tract and represents one of the most common bacterial infections (1).

Clinically UTIs can manifest as urethritis, cystitis or pyelonephritis.

EAU classification of UTIs includes uncomplicated UTIs, complicated UTIs, recurrent UTIs (rUTIs), catheter associated UTIs and urosepsis.

Recurrent UTIs (rUTIs) are UTIs presenting with a frequency of at least three times/year or twice in the last six months.

In this paper we want to evaluate the possible effects of a phytotherapeutic product (D-Mannose, Ellirose™ and Lactobacillus Plantarum) in recurrent UTIs.

Almost half of all women will experience at least one episode of cystitis during their life (1).

Risk factors include sexual intercourses, use of spermicide, a new sexual partner, a mother with an history of UTIs and an history of UTI during childhood (2).

The spectrum of etiological agents includes E. Coli in 70-95% of cases, Staphylococcus Saprophyticus in 5-10% of cases and occasionally other enterobacteriaceae such as Proteus Mirabilis and Klebsiella.

The most important reservoir of uropathogenic E. Coli is the human intestine. Bacteria can be considered normal inhabitants of the gastrointestinal tract where they play an important role in the maintenance of health and in development of diseases (3).

E. Coli and other Bacteria can reach the number of 1014 in the colon and more than 1000 bacterial species are found in the intestine. In the human intestine E.Coli can become a resident bacteria, survive and persist for many years and finally give rise to bacterial foci, acting like reservoir of UPEC (Uropathogen E. Coli) able to cause UTIs (4).

Urogenital colonization by E.Coli is feasible to a series of predisposing factors, hygiene habits and sexual practices (5).

In most cases female urethra gets infected by fecal material containing uropathogenic bacteria according with the short distance between female urethra and the anus that facilitates contamination (5).

After colonization the next step is adhesion of the bacteria to epithelial cells of urogenital mucosa.

This adhesion is mediated by special hair-like virulence factors of bacteria called fimbriae, particularly type 1 and the P- fimbriae (6).

Intestinal epithelium has several defense mechanisms wich include mucociliary clearing, rapid cell turnover, epithelial cell exfoliation and shedding and finally the urinary flow (7).

However some bacteria have developed mechanisms to bypass host defense mechanisms.

In particular uropathogenic E. Coli expresses special proteins that bind to same host receptors called CEA-related cell adhesion molecules increasing bacteria's stickiness to the infected cells (7). Also blocking epithelial cell exfoliation and shedding Bacteria facilitate the development of rUTIs.

Last step consists in invasion of nearby cells and tissues. Uropathogenic E.Coli can also replicate within the bladder cells and form intracellular Bacteria foci wich have been correlated to rUTIs.

Recurrent UTIs consist in recurrences of uncomplicated and/or complicated UTIs with a frequency of at least 3 UTIs /year or 2 UTIs in the last 6 months.

RUTIs include lower tract infections (cystitis) and upper tract infections (Pyelonephritis) and are very common in general population, in particular in women.

About 20-30% of women with a first UTI will have a recurrence and 5% will have chronic recurring infections (8).

Diagnosis should be confirmed by urine culture: a colony count of 10.000 cfu/mL of uropathogens is microbiologically diagnostic in women who present lower urinary tract symptoms as dysuria, frequency, urgency and suprapubic pain with absence of vaginal discharges or irritation.

Risk factors can be considered for young premenopausal women and for post-menopausal and elderly women.

For young women risk factors are sexual intercourses, use of spermicide, a new sexual partner, a mother with a history of UTIs during her childhood and blood antigen secretory status.

For elderly women can be considered as risk factors history of UTIs before menopause, urinary incontinence, atrophic vaginitis, cystocele, increased post-void urine volume, blood group, antigen secretory status, urine catheterization, functional status and deterioration in elderly institutionalised women.

Prevention of rUTIs consists in avoidance of risk factors and prophylaxis with antimicrobial and non antimicrobial measures, unfortunately associated with onset of resistance to the antibiotic.

Non antimicrobial prophylaxis includes administration of probiotics, Cranberry and D-Mannose.

In particular, Lactobacillus probiotics and D-Mannose are included the preparation associated to Ellirose™.

Methods

The product is a phytotherapeutic composed of D-Mannose (1000 mg), Ellirose™ (200 mg) and Lactobacillus Plantarum Lp - 115 (1 mld u.f.c.).

It can be used in non antibiotic prophylaxis of rUTIs and its posology is 2 sachets daily (1 in the morning and 1 in the evening) in presence of urinary symptoms or after an invasive out-patient examination or 1 sachet daily in other clinical situations.

It can be administrated for prolonged time for its generally minimal side-effects.

Its clinical use is based on molecular effects of its components and on their synergistic effects.

In order to understand how this dietary supplement is prescribed, in wich kind of patients and clinical situations, we drafted a quick and concise survey.

We administrated this questionnaire to 12 residents in Urology at the University of Modena and Reggio Emilia and to 32 urologists working in the provinces of Modena, Reggio Emilia and Parma.

The questionnaire was answered by mail or trough direct anonymously compilation.

Investigational survey on a phytotherapeutic product made of D-Mannose, Ellirose™ and Lactobacillus Plantarum

1) How many times have you diagnosed rUTIs during the last month?
- 0-3 times
- 4-7 times
- ->8 times

2) Do you trust in alimentary supplements (non pharmacological therapy) for the treatment of urological disorders?
- yes
- no

3) How many times have you prescribed the phytotherapeutic product during the last month?
- 0
- 1-5
- ->5

4) For wich urological diseases have you prescribed the phytotherapeutic product?
- None
- rUTIs as non antimicrobial prophylaxis
- others

5) Have you noticed an improvement in the quality of life of patients affected of rUTIs with the phytotherapeutic product as non antimicrobial prophylaxis?
- I do not use
- yes
- no

6) Have you noticed an improvement of urinary symptoms as dysuria, burning, frequency, urgency and suprapubic pain?
- I do not use
- yes
- no

7) Do you administrate the phytotherapeutic product after cistoscopy or invasive urodynamic exam?
- I do not use
- yes
- no

8) Have patients reported occurrence of gastric side effects after assumption of the phytotherapeutic product?
- I do not use
- yes
- no

9) Have patients reported occurrence of other side effects after assumption of the phytotherapeutic product?
- I do not use
- yes
- no

10) Is the cost of the phytotherapeutic product affordable for the majority of patients?
- I do not use
- yes
- no

Results

All the 44 physicians completed the questionnaire in all its parts demonstrating that the phytotherapeutic product made of D-Mannose, Ellirose™ and Lactobacillus Plantarum is a well known product in this area, with a wide diffusion between both young and senior urologists.

The 61% of physicians have diagnosed rUTIs in 3-6 patients during a month, and 7% of them in more than 6 patients during the same period of time. By these result rUTIs appear as common pathological conditions.

16% of physicians surveyed didn't believe in use of dietary supplements for treatment of any medical condition so they refused the use of the product whereas 59% prescribed it at least 1 time a month and 14% prescribed it more than 5 times.

The majority of physicians who administered the phytotherapeutic product prescribed it correctly for rUTIs.

43% administrated the product after out-patient invasive examinations as cistoscopy and urodynamic exam for UTIs prevention.

20% of physicians have not noticed a significant improvement in patient's QoL (Quality of Life) suffering from rUTIs, while the 55% had evidence of clear ameliorations.

Furthermore 48% also reported a significant effect for the improvement of urinary symptoms of patients.

No gastric or general side effects have been noticed during the administration period.

Finally the cost of integrator has reported affordable for the great majority of patients.

Results of investigational survey on a phytotherapeutic product made of D-Mannose, Ellirose™ and Lactobacillus Plantarum

1) How many times have you diagnosed rUTIs during the last month?
- 0-3 times: A 14, 32%
- 4-7 times: B 27 61%
- >8 times: C 3 7%

2) Do you trust in alimentary supplements (non pharmacological therapy) for the treatment of urological disorders?
- yes: A 37, 84%
- no: B 7, 16%

3) How many times have you prescribed phytotherapeutic product during the last month?
- 0: A 12, 27%
- 1-5: B 26, 59%
- >5: C 6, 14%

4) For wich urological diseases have you prescribed phytotherapeutic product?
- None: A 9, 20%
- rUTIs as non antimicrobial prophylaxis: B 33, 75%
- others: C 2, 5%

5) Have you noticed an improvement in the quality of life of patients affected of rUTIs with phytotherapeutic product as non antimicrobial prophylaxis?
- I do not use: A 11, 25%
- yes: B 24, 55%
- no: C 9 20%

6) Have you noticed an improvement of urinary symptoms as dysuria, burning, frequency, urgency and suprapubic pain?
- I do not use: A 11, 25%
- yes: B 21, 48%
- no: C 12, 27%

7) Do you administrate phytotherapeutic product after cistoscopy or invasive urodynamic exam?
- I do not use: A 11, 25%
- yes: B 19, 43% b
- no: C 14, 32%

8) Have patients reported occurrence of gastric side effects after assumption of phytotherapeutic product?
- I do not use: A 11, 25%
- yes: B 0,
- no: C 33, 75%

9) Have patients reported occurrence of other side effects after assumption of phytotherapeutic product?
- I do not use: A 11, 25%
- yes: B 0,
- no: C 33, 75%

10) Is the cost of phytotherapeutic product affordable for the majority of patients?

- I do not use: A 11, 25%
- yes: B 32, 73%
- no: C 1, 2%

From the investigational survey on the phytotherapeutic product can be assumed that it is a dietary supplement widely used by the urologists in our region.

The absence of side effects makes it easily and safety administrable for long period especially for non-antimicrobial prophylaxis in rUTIs without risk of development of any antibiotic resistances.

The number of physicians surveyed is limited but data reported on the amount of prescriptions and on patient feedback indicate that phytotherapeutic product has a positive impact on clinical course of recurrent UTI.

D-Mannose contained has an important role in prevention of rUTIs. It is a sugar with a relevant role in human metabolism, especially in glycosylation of certain proteins.

The supposed mechanism of action is inhibiting bacterial adherence to uroepithelial cells (9).

B. Kranjcec et al. Investigated the role of D-Mannose in prevention of rUTIs in women with history of rUTIs initially treated with antibiotic therapy. These 308 women were randomly allocated in 3 groups. The first group (n=103) received prophylaxis with 2 gr of D-Mannose powder in 200 ml of water daily for 6 months.

The second group (n=103) received 50 mg Nitrofurantoin daily and the third group (n=102) did not received any products.

Overall 98 patients (31,8%) had rUTIs: 15 (14.6%) in the D-Mannose group, 21 (20.4%) in Nitrofurantoin group and 62 (60.8%) in no prophylaxis group.

Patients in first two groups had a significantly lower risk to develop rUTIs compared to patients in no prophylaxis group (RR 0.239 and 0.335, $p < 0.0001$).

Moreover patients in D-Mannose group had a significantly lower risk of side effects compared to patients in Nitrofurantoin group (RR 0.276, $p < 0.0001$) (10).

H. Sabdariffa is a common herbal drink consumed both hot and cold by people around the world and used in traditional medicine for the treatment of hypertension and UTIs.

The infusion is usually called karkade or red tea made from the calyces of the H. Sabdariffa. The red calyces area rich in organic acids as ascorbic acid, minerals, anthocianins, other phenolic components and antioxidants agents.

Issam Alshami et al. in their study wanted to asses the effectiveness of H. Sabdariffa extract in inhibiting the biofilm forming capacity of uropathogenic bacteria.

The antimicrobial activity of the H. Sabdariffa extract was evaluated on 8 different uropathogenic bacteria: 6 Escherichia Coli and 2 Klebsiella Pneumoniae isolates collected from patients with rUTIs. Extract inhibited biofilm production of all the isolates supporting the effective potential of H. Sabdariffa extract to prevent rUTIs (11).

The use of probiotics in prophylaxis is not yet supported by clinical studies showing convincing benefits but the role of Lactobacilli to maintain a normal vaginal flora equilibrium and to interfere with adherence, grown and colonization of uropathogenic bacteria seem suggest a use in prevention of rUTIs (12).

Conclusions

Many studies in Literature have shown that D-Mannose and H. sabdariffa (Ellirose™) reduce the risk of development of rUTIs opposing to colonization and proliferation of uropathogenic bacteria in urinary tract.

Moreover the absence of several side effects during prolonged prophylaxis is really considerable.

Our investigational survey has shown that this phytotherapeutic product is well-known and has a proved positive impact.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Influence of various environmental factors on the growth of children and adolescents in Jeddah, Kingdom of Saudi Arabia

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Summary. *Objectives:* To evaluate the association between various environmental factors and the anthropometric measurements of children and adolescents. *Methods:* This retrospective studywasperformed from September 2017 to April 2018 and included 393 children aged 2-18 years. Data were extracted through patient and/or parentinterviews and from medical records of endocrine ambulatory clinics. *Results:* Among underweight children,the proportion of mixed-fed children was the highest, and among normal weight children, most were either bottle-fed or mixed-fed. Most overweight children were mixed-fed, and most obese children were breastfed. Underweight status was noted most commonly in children playing videogames for <2 hours/day, followed by those playing>4 and 2-4 hours/day. Normal weight was noted most commonly in those playing for >4 hours/day, followed by those playing <2 and 2-4 hours/day. Overweight status was noted most commonly in those playing videogames for >4 hours/day, followed by those playing 2-4 and <2 hours/day. Most children playing videogames for <2 hours/day were obese. Mean BMIs were the highest in those who exercised 1-2 times/week. P-values for the association between passive smoking indoors and BMI, weight, and height were 0.045, 0.150, and 0.854, respectively. Regarding socioeconomic status, log BMI values were 1.22, 1.23, and 1.26 in low-, medium-, and high-income families, respectively (P-value, 0.001). *Conclusion:* Children who were bottle-fed in their first year of life, played video games >2 hours/day, did not exercise regularly, were exposed to indoor passive smoking, and had a high socioeconomic status had a higher BMI and weight than their counterparts. (www.actabiomedica.it)

Key words: growth, environmental factors, children, adolescent, height

Introduction

Monitoring a child's growth is an adequate approach for measuring the health and nutrition status of children, with normal growth indicating good health and nutrition conditions (1). Over the past decade, a significant increase in the body mass index (BMI) of children in Jeddah has been noted (2). The estimated average prevalence of obesity in Jeddah is 17.8% (3). Recent studies conducted in three different regions (central, northern, southwestern) of Saudi Arabia showed a noticeable difference in growth rates among children and adolescents (4), which variation may be attributed to genetic heterogeneity and prenatal and postnatal environmental effects (5). Global research supports the putative influence of environmental factors onchildren's growth (4, 5).

Feeding type is associated with growth in children. Breastfed children exhibit lower body weight and height than their bottle-fed counterparts (6). The type

of foods consumed (7, 8); the socioeconomic status (SES) of parents, family size, and maternal education (9); and other environmental factors such as the use of electronic devices, sleep deprivation, participation in physical exercise (10-12), and exposure to smoking (13) all affect children's growth and development.

However, studies comprehensively investigating the relationship between various environmental factors and growth are lacking. Thus, we aimed to identify these factors and their relationship with growth among children and adolescents in Jeddah, Kingdom of Saudi Arabia.

Methods

Study Design

This retrospective cohort study was conducted in the departments of pediatrics and endocrinology at various ambulatory pediatric endocrine clinics in Jeddah city, Saudi Arabia, between September 2017 and April 2018.This study was conducted in accordance with STROBE (EQUATOR) guidelines.

Participants

The participants were healthy 393 children and adolescents aged between 2 and 18 years. Those below the age of 2 years and above the age of 18 years were excluded from this study. Children with chronic conditions and medical syndromes were also excluded.

Data Collection

Data was collected from interviews with patients and/or their parents and by reviewing clinical medical records through the Phoenix system.

Anthropometric Measurements

Pediatric measurements including height, weight, and BMI were obtained from the medical records of the patients. The standard deviation (SD) values for height and weight were calculated using the growth calculator software "http://growthcalc.chip.org/".

Data variables

Various environmental factors were reviewed, including the type of child nutrition during the first year of life (breast, bottle, or mixed feeding). Data on daily physical activity levels (exercise duration and number of days of exercise per week), time spent on electronic devices and video games per day, and duration of sleep were also reviewed. Additionally, we also reviewed data on the socioeconomic class, which was assessedusingfamily income, number of rooms in a family's residence, and number of family membersand exposure to passive cigarette smoking (evaluated by gauging whether family members smoked indoors or outdoors, and the number of smokers in the household).

Definitions

BMI is the best measure for weight categorization and is defined as the weight in kilograms divided by the height in square meters. Children were grouped according to their BMI values as normal (5^{th} to < 85^{th} percentile), overweight (85^{th} to < 95^{th} percentile), obese (≥ 95th percentile), or underweight (< 5th percentile) using World Health Organization data (14). The use of video games and electronic devices was measured based on whether children played them for more or less than 2 hours per day (15). The average duration of physical activity and exercise among children is considered to range from 30 to 60 min per day (16). SES was measured by the monthly income of the family, family size, and number of rooms in the house. The monthly income was defined as 'low' if it was 2000-4000 Riyals, 'middle'if it was 5000-9000 Riyals, and 'high' if it was higher than10,000 Riyals (17). Family size was defined as 'small' if there were 3 or fewer members, 'medium'if there were 4-6 members, and 'large' if there were more than 6 members. Houses with 3 rooms or fewer were defined as 'small', those with 4-6 rooms were defined as 'medium', and those with more than 6 rooms were defined as 'large'. The appropriate sleep duration per day for toddlers is between 11 and 14 hours, for preschoolers between 10 and 13 hours, for school-aged children between 9 and 11 hours, and for teenagers between 8 and 10 hours (18). Exposure to smoking is thought to affect children's growth; light

smoking was defined as the smoking of 1 to 9 cigarettes per day, while heavy smoking was the smoking of more than 10 cigarettes/day, according to Ferris et al. 1985 (19).

Ethical considerations

Approval for this study was obtained from the Institutional Review Board of King Abdulaziz University Hospital before study implementation. Confidentiality of patient data was maintained according to the Declaration of Helsinki.

Statistical analysis

Data were analyzed using the Statistical Package for Social Science (IBM SPSS) version 22. The sample included 393 children treated at medical clinics in Kingdom of Saudi Arabia, Makkah Region. The relationship between environmental factors that affect growth and body measurements was tested using various statistical methods.

One-way analysis of variance and an independent sample t-test were used to test the relationship between environmental factors and body measurements, under the assumption that the continuous data followed a normal distribution depending on the normal curve. Spearman's correlation test was used for ordinal variables to test the relation of environmental factors such as lifestyle and eating habits with BMI, weight, and height. Descriptive statistics were reported as proportions for qualitative variables and statistics were reported as the mean and SD for quantitative variables. Results were considered significant at P <0.05.

Results

This study included a total of 393 children and adolescents aged between 2 and 18 years. A total of 211 (53.7%) of them were boys, and 182 (46.3%) were girls.

Of all the children who were screened, 100 (25.4%) were bottle-fed, 129 (32.8%) were breastfed, and 164 (41.7%) received mixed feeding (bottle-feedingand breast feeding).

The relationship between feeding types and body mass index (BMI) categories is demonstrated in Table 1. Height categories are explained in Figure 1, and weight categories in Figure 2.

Forthe feeding types, the p-values for group differences were 0.381, 0.018, and 0.009, respectively, for height, weight, and BMI.

Table 1. Weight categories among the feeding types in the first year

Body mass index categories	Type of feeding		
	Bottle feeding	Breastfeeding	Mixed feeding
Underweight	59 21.7%	89 32.7%	124 45.6%
Normal weight	31 33.7%	30 32.6%	31 33.7%
Overweight	7 31.8%	7 31.8%	8 36.4%
Obesity	3 60.0%	2 40.0%	0 0.0%
Total	99 25.3%	129 33.0%	163 41.7%

P-value= 0.018

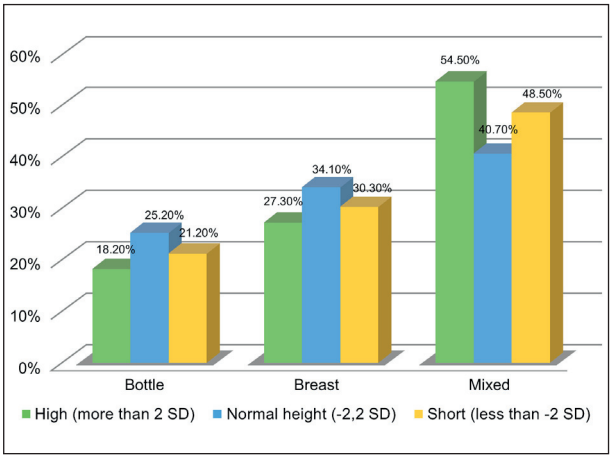

Figure 1. Height according to feeding types in the first year
Children who were breastfed or received mixed feeding were taller than bottle-fed children
SD, standard deviation

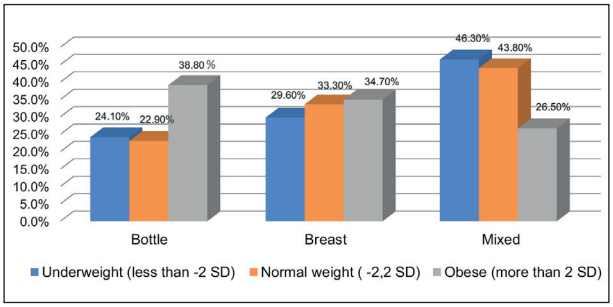

Figure 2. Weight according to types of feeding.
children who were bottle-fed had higher weight values than children who were breastfed or received mixed feeding
SD, standard deviation

Regarding video games, 338 (86.2%) children played video games, and 54 (13.8%) did not. A total of 121 (35.8%) children played video games for less than 2 hours per day, 84 (24.9%) played video games for 2-4 hours, and 133 (39.3%) played video games for more than 4 hours. In 127 (34.5%) children, video games interfered with the daily routine, and in 241 (65.5%) children, video games did not interfere with the daily routine.

Details ofBMI categories among children who played video games are demonstrated in Figure 3. The P-values for the weight and height categories were 0.870 and 0.244, respectively.

Details regarding height categories are provided in Figure 4, and those regarding the association between the weight categories and time spent playing videogamesare provided in Figure 5.

Regarding physical activity, 51 (35.4%) children exercised 1-2 times per week, 39 (27.1%) exercised 3-5 times per week, and 54 (37.5%) exercised every day, and their mean BMI values were 19.25, 19.0, and

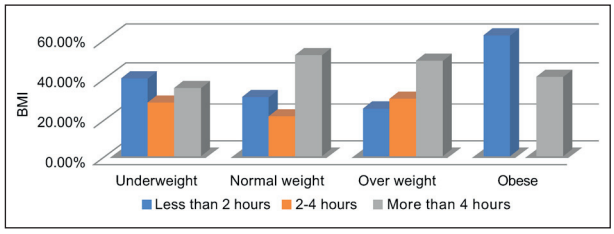

Figure 3. BMI according to timespent playing video games
Children who played video games for less than 2 hours/day had lower log BMI values than those in the other groups
BMI, body mass index

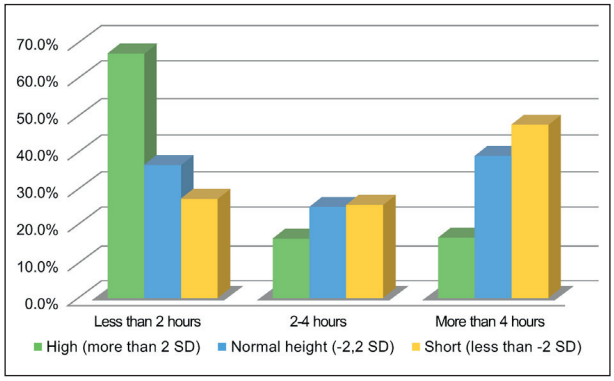

Figure 4. Height according to hours spent playing video games
Children who played video games for more than 4 hours/day were shorter than their counterparts in the other groups
SD, standard deviation

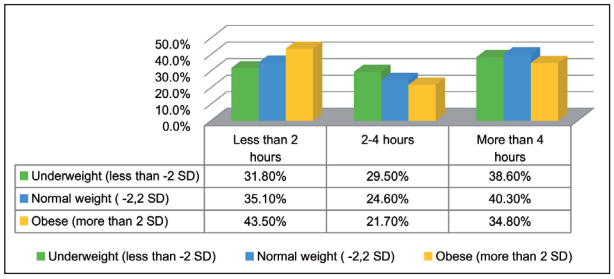

Figure 5. Weight according to hours spent playing video games
children who played video games for less than 2 hours/day were underweight or had normal weight, while children who played for 2 hours and more, were overweight or obese
SD, standard deviation

17.40, respectively (P-value of 0.037). The P-value for differences in height was 0.278. Among children who exercised daily, 16 (11.2%) exercised for less than 30 min/day, 69 (48.3%) for 30-60 min/day, and 58 (40.6%) for more than 60 min/day. Their mean weights were 1.3275, 0.0501, and -0.1740, respectively (P-value of 0.029). Forheight, the SDs of the means were -0.1531, -0.5438, and -0.8260, respectively (P-value of 0.251).

As for sleep duration and its effect on growth, 6 (50%) toddlers slept for less than 11 hours/day, and 6 (50%) slept for 11-14 hours/day; 44 (48.4%) preschoolers slept for less than 10 hours/day, and 47(51.6%) for 10-13 hours/day. A total of 76 (35.7%) school-aged children slept for less than 9 hours/day, 132 (62%) for 9-11 hours/day, and 5 (2.3%) for more

than 11 hours/day. Eleven (15.1%) teenagers slept for less than 8 hours/day, 50 (68.5%) for 8-10 hours/day, and 12 (16.4%) for more than 10 hours/day. The mean BMI value of children who slept <8h was 1.2384, for 8-10h was 1.2412, and for >10 h was 1.2195 (P-values of 0.225, 0.730, and 0.512 for BMI, height, and weight, respectively).

Regarding passive smoking exposure, 274 (69.7%) children came from families with no smokers, and 96 (24.4%) had one parent who was a smoker. Both parents of11 children (2.8%) smoked and more than two family members of 12 (3.1%) children were smokers. As for the number of cigarettes smoked per day, 32(27.4%) smokers smoked 1-9 cigarettes per day, 46 (39.3%) smoked more than 10 cigarettes per day, and 39 (33.3%) smoked shisha every day. A total of 68 (56.2%) family members smoked inside the house, and 53 (43.8%) smoked outside the house. The log BMI value for passive smoking inside the house was 1.2656, with P-values of 0.045, 0.150, and 0.854 for BMI, weight, and height, respectively, while the log BMI for passive smoking outside the house was 1.2307. Regarding the number of smokers in the house, the log BMI values were 1.23, 1.31, and 1.27, respectively, in cases with no smokers in the house, cases in which both parents were smokers, and cases in which there were more than 2 smokers in the house, with a P-value of 0.017. The P-values for height and weight were 0.872 and 0.139, respectively.

As for SES and its relationship with growth, 96 (24.4%) of the participants' families had a low income (2000-4000SR), 170 (43.3%) had a medium income (5000-9000SR), and 127 (32.3%) had a high income (>10,000). This was reflected in the log BMI values of 1.22, 1.23, and 1.26, respectively, with a P-value of 0.001. The height P-value was 0.259 and weight P-value was 0.801.

A total of 131 (33.4%) families lived in a property with 3 rooms or fewer, 207 (52.8%) had 4-6 rooms, and 54(13.8%) had more than 6 rooms. The P-values were 0.0001, 0.253 and 0.287 for BMI, weight, and height, respectively. As for family size, 34 (8.7%) children had 3 or fewer members in their families, 269 (68.4%) had 4-6 members, and 90 (22.9%) had more than 6 members. Families with 3 or less members had a log BMI= 1.19, those with 4-6 members had a log BMI=1.23, and those with more than 6 members had a log BMI=1.26, with a P-value= 0.001. The weight and height P-values were 0.691 and 0.995, respectively.

Discussion

Types of Feeding

Previous studies have shown the relationship between environmental factors and their effect on children's growth. The maintenance of a good nutritional status is crucial to the healthy growth of children and adolescents. Nutrition during the first year of life critically affects children's growth later in life. The provision of nutrition in the first year of life is divided into: breastfeeding, bottle feeding and mixed feeding. Many studies have shown that children who have been breastfed have a lower body weight and shorter body length than their bottle-fed counterparts (6, 20, 21). Similarly, in our study, we found that children who were bottle-fed had higher BMIand weight values than children who were breastfed or received mixed feeding; there was no significant relationship between the height categories and feeding types.

Video Games

In Saudi Arabia, with the increase in the number of children with sedentary lifestyles and the time spent playing video games, children spend less time exercising, playing sports, or performing other activities;this affects their growth negatively by increasing obesity rates and therate of sleep deprivation (10, 11, 22). In Jeddah, in 2016, studies found that 68.4% of children played video games for 2 hours or more and 48% exercise for less than 30 minutes per day. An increased BMI was found among children who spent ≥2 hours per day on electronic devices (15). In comparison, 38.90% of children who played video games for less than 2 hours/day were underweight or had anormal weight, while among children who played for 2 hours and more, 47.60% were overweight and 40% were obese. An increased BMI was observed,over the years, among children who spent more than 2 hours/day playing video games.

Sleep Duration

Several studies have demonstrated the relationship between sleep duration and weight. In Korea, a study indicated that a longer sleep duration in adolescents was associated with a lower BMI (12). Another study showed that children who had a short sleep duration had an increased BMI (23). However, our study revealed no significant association between BMI, weight and height categories, and sleep duration.

Exercise and Physical Activity

In 2013, a study conducted by Al-Ghamdi in Riyadh, showed positive association between exercise duration and BMI, with BMI increase observed with a decrease in the time spent on physical activities. This study included 397 students, and a notable proportion of children who did not exercise or exercised for less than 30 minutes were either obese or severely obese (16). These findings are similar to our results, in which children who exercised every day for 60 minutes or more had a lower body weight. In our study,a significant association was observed between decreased levels of physical activity and increased body weight and BMI.

Passive Smoking

Data from the National Study of Health and Growth in England and Scotland indicate that children's height is associated with the number of smokers in their households, taking birth weight into account. Additionally, an analysis of data on 5,903 children from a study of primary schools in England and Scotland in 1982 showed that the number of cigarettes smoked by parents at home was significantly linked to the attained height of their children (13). The prevalence of smoking in Saudi Arabia ranges from 11.6-52.3% among adults, indicating that many children are at the risk of being exposed to indoor pollutants (24). Our study revealed that children with family members who smoke indoors have a higher BMI than children with family members who smoke outdoors. No effect was observed on weight or height individually. Regarding the number of passive smokers in the house, a high log BMI mean was observed when both parents were smokers, compared to cases in which none or one of the parents was a smoker. No significant association was observed between the number of smokers in the house and the height or weight standard deviations.

Socioeconomic Status

The relationship between parents' SES, and children's weight is complicated. Previous studies have shown that a low SES may lead to risky behaviors (lack of physical activity, poor diet, and sedentary lifestyle) and can result in obesity and overweight (25, 26). Similarly, children living in relatively wealthy families are likelyto receive more attention and specialized care from parents who offer a variety of cultural and physical activities and educate them on the virtues of a healthy diet (27).

Family Income

In another study performed on 1,072 children in Saudi Arabia, 95% of the obese children came from families with high incomes (28). In less industrialized countries, overweight and obesity are prevalent in families with higher incomes (29). In our previous study, we demonstrated a higher risk of overweight in relation to high family income (30). This could be attributed to the fact that children from high-income families have increased accessibility to fast food and dine out multiple times a week while families with low incomes tend to depend more on homemade meals. The present study's findings align with those ofthe previous studies because higher BMI values were associated with a higher SES, as we observed a directrelationship between SES (in terms of family income and number of rooms) and BMI.

Family Size

Children in households with more than 4 members were 1.35 times likelier to be underweight than families with 1-4 members (31). We found that children from families with 4-6 members or more were likelier to have a higher BMI than their counterparts with 3 or fewer family members; this finding contradicts that of the previous study.

Study limitation

Our study is limited by the relatively small sample size; therefore, our findings may not be generalizable to the general population of Saudi Arabia. Future studies should include a larger sample size, along with a detailed survey to help understand the magnitude of each factor individually.

Conclusion

A notable increase in both BMI and weight was found to be more significantly associated with bottlefeeding thanwith breastfeeding or mixedfeeding. Moreover, spending time playing video games, lack of exercise, exposure to indoor passive smoking, and high SES were also associated with increased BMI and weight.

Recommendation

We recommend the provision of global education for parents, focusing on the benefits of breastfeeding, indoor physical activity, limiting the use of electronic devices and videogames to less than 2 hours/dayand discouraging exposure to indoor passive smoking,to optimize the growth of children and adolescents.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Effect of growth hormone treatment on children with idiopathic short stature (ISS), idiopathic growth hormone deficiency (IGHD), small for gestational age (SGA) and Turner syndrome (TS) in a tertiary care center

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Summary. *Objectives:* To assess the long-term effect of growth hormone (GH) therapy in a large cohort of short children with different etiologies. *Patients and Methods:* We evaluated retrospectively the anthropometric data of 252 short children [height SDS <-2: 154 children with growth hormone deficiency (GHD), 63 with idiopathic short stature (ISS), 26 with SGA, and 9 with Turner syndrome (TS)] who were treated, in our center, with GH between 1-2007 and 1-2018. Before and during recombinamt growth-hormone (recGH) treatment, auxological parameters including height (Ht), weight (Wt), Ht - Z score (HtSDS), body mass index (BMI) and BMISDS were recorded every 6 months; bone age (BA) was assessed every 12 months. *Results:* At the end of first year of rhGH therapy and after an average of 3 years treatment all groups of short children had significant increase in HtSDS, which was higher in GHD compared to other groups. Children with GHD, SGA, ISS and TS increased their HtSDS by an average of 2.2, 1.46, 0.6 and 0.99 SD, respectively at the end of follow up period (for all groups, p: <0.001). The bone age/chronological age (BA/CA) ratio did not differ significantly among ISS, GHD and SGA groups after GH therapy. The HtSDS gain was higher in children with GHD compared to other ISS, SGA and TS groups (p:< 0.01; p: 0.015 and p: 0.029, respectively). HtSDS improvement occurred during the first 3 years of rhGH therapy. The BMISDS increased significantly in children with GHD, after 3 years of rhGH therapy (p: < 0.001). After rhGH treatment, the BMISDS decreased significantly in children with ISS and SGA (p: < 0.01 and < 0.001, respectively) but did not change in children with TS (p: 0.199). *Conclusions:* Children with GHD, SGA, ISS and TS exhibited significant increases in HtSDS when treated with rhGH for 3 years. The HtSDS gain was higher in children with GHD compared to other groups. (www.actabiomedica.it)

Key words: linear growth, HtSDS, GH deficiency (GHD), idiopathic short stature (ISS), small for gestational age (SGA), Turner syndrome (TS), recombinant GH therapy (rhGH)

Introduction

Plentiful supply of recombinant growth hormone (rhGH), acquired via recombinant DNA technology, enabled the expansion of its use beyond replacement of deficient growth hormone (GHD) including short children with non-GH deficiency disorders. Short stature is defined as a height of less than -2 standard deviation (SDs) compared to the mean height at the corresponding age and sex.

Idiopathic short stature (ISS) is a short stature condition in children with a normal birth weight,

normal body proportions, normal GH response to stimulation tests, height SDS corresponds to mean parental SDS, and no identified cause for their short stature. However, ISS children represents a heterogeneous group of children with many non-specific causes of short stature. Children with familial short stature and constitutional delay of growth and puberty are included in the ISS category. In 2003, the United States Food and Drug Administration approved the use of rhGH for children with a height SD score (HtSDS) of less than -2.25 and a short predicted adult height (PAH) (1-5).

In addition, clinical data showed that rhGH is an effective therapy for short children who are born small for gestational age (SGA). Therefore, short children born SGA who fail in their catch-up growth by 2-4 years of age are candidates for rhGH therapy. It has been shown that, in SGA children, the response to rhGH therapy varies with GH status. Therefore, it has been recommended that GH status be assessed in patients born SGA to optimize rhGH treatment (6,7).

Several studies have shown that rhGH therapy increases adult stature in TS, and this therapy is approved by the U.S. Food and Drug Administration (FDA) and other regulatory agencies worldwide with variable results on final adult height (8,9).

Few previous clinical data have been compared the growth response to long-term rhGH therapy in short children with ISS, TS and SGA to those with isolated GHD. Here, we present and compare the effects of GH treatment in children with ISS, TS and SGA in comparison to those with GHD, treated in a tertiary care center (10).

Patients and methods

1. Patients

We retrospectively reviewed the medical records of 252 children with short stature (height SDS <-2) who were diagnosed and treated, between January 2007 till January 2018, in our tertiary care center with rhGH. Children with organic brain lesions, systemic diseases, or syndromes that result in growth disorders (apart from TS) were excluded.

Idiopathic short stature was diagnosed, in whom with no identifiable disorder, when the height of the child was more than 2 SD below the corresponding mean height for age and sex (11).

Small for gestational age (SGA) was defined as a birth weight below the 10th percentile for the gestational age, compared to a gender-specific reference population (12,13).

Growth hormone deficiency (GHD) was diagnosed when the child height was below -2 SD and or when the annual growth velocity was < -1 SD or the standing height was < −1.5 SD below the mid-parental height, in addition to a defective peak response to GH provocative testing (below 10 μg/L) associated to an insulin growth factor -1 (IGF1) level < -2 SD (14,15).

Turner syndrome (TS) was diagnosed when the X chromosome was partially or completely missing in a female (16).

All children included in the study (apart from SGA) had a normal birth weight. Among the short statured children, conventional GH provocation tests, using oral clonidine and subcutaneous glucagon, were performed to classify them as either ISS or GHD. If the magnetic resonance imaging (MRI) of hypothalamic-pituitary region was found normal in GHD children, a diagnosis of idiopathic GHD (IGHD) was made (17-21).

Based on the previous definitions, our patients were grouped into the following diagnostic categories: 154 children with GHD, 63 children with ISS, 26 children with SGA, and 9 patients with TS.

Exclusion criteria included children with systemic, metabolic or other endocrine diseases or other hormonal deficiencies (thyroxine, cortisol), insulin-dependent diabetes, chronic inflammatory and infectious disorders, anemia, and genetic syndromes (other than TS) or bone disorders.

All children included in the study had normal hemogram, renal and hepatic functions and calcium homeostasis. Circulating concentrations of free thyroxine (FT4), thyroid stimulating hormone (TSH), early morning plasma cortisol and fasting blood glucose (FBG) were normal. None of the children, included in the study, was receiving medications.

2. Methods

Before and during rhGH therapy, auxological and biochemical parameters including: height (Ht), weight (Wt), Ht-Z score (HtSDS), body mass index (BMI) and BMISDS were recorded every 6 months; bone age (BA) was assessed every 12 months. BA was evaluated using the Greulich-Pyle method, while the predicted adult height (PAH) was calculated by Bailey-Pinneau method.

Annual and total increment ratios of HtSDS were calculated over the period of full years of rhGH therapy. rhGH treatment was given at a dose 0.03 to 0.05 mg/kg/day in children with

GHD, 0.025 to 0.05 mg/kg/day to those with ISS and SGA and 0.04 to 0.06 mg/kg TS subjects. The rhGH dosage was adjusted according to the children's insulin like growth-factor 1 (IGF1) level in order to avoid levels > 2 SD (22-27).

3. Statistical analysis

Statistical analysis was performed by using SPSS, 21th edition, statistical Package. All data are expressed as mean ± SD values. ANOVA test was used to compare growth data among the 4 study groups. Paired t-test was used to compare HtSDS data after versus before rhGH treatment in each group and non-paired t test to compare HtSDS changes among different groups. Mann-Whitney *U*-test was applied to compare the differences of numerical variables between the groups at each time and chi-square test or Fisher exact test was performed to compare the frequencies between groups. Wilcoxon signed rank test was applied to compare differences of the variables within the groups at each time. A *P*-value of 0.05 or less was considered statistically significant. In multiple comparisons between all times, the Bonferroni correction was applied and a *P*-value of 0.05 or less was considered statistically significant.

Results

A total of 252 short stature children (height SDS <-2) were diagnosed and treated with rhGH in our tertiary center. The study included: 154 children with GHD, 63 with ISS, 26 with SGA, and 9 with TS. Table 1 shows the anthropometric data registered in the 4 studied groups, before rhGH treatment and at the last examination.

During the treatment period with rhGH, none of our patients with GHD, ISS and SGA had early puberty (before the age of 8 years in girls and 9 years in boys) or delayed puberty (breast development after 13 years in girls and testicular enlargement after 14 years in boys).

Before and during the treatment, all children had normal fasting BG [less than 100 mg/dL (5.6 mmol/L)] and thyroid function. Two children with GHD developed pedal edema and 3 had local allergic manifestations at the site of rhGH injection. One patient with SGA developed symptoms of high intracranial pressure (headache and vomiting) that disappeared with discontinuation of rhGH therapy. Two children complained of non-specific arthralgia that did not necessitate discontinuation of therapy.

Table 2 presents the effect of rhGH therapy in the 4 study groups and duration of their treatments. After an average of > 3 years of treatment, children with GHD increased their

HtSDS by an average of 2.2 SD. Those with TS and ISS increased their HtSDS by an average of 0.99 SD and 0.65 SD after an average of 3.5 and 7.5 years, respectively. Children with SGA increased their HtSDS by an average of 1.46, after 3.5 years of treatment.

The ANOVA test showed a significant difference in the HtSDS and BMISDS changes among the 4 groups. The bone age/chronological age (BA/CA) ratios were not significantly different between the ISS, GHD and SGA groups, after 1 and 3 years of rhGH therapy.

Table 3 compares the growth responses to rhGH therapy in GHD versus ISS groups. Both groups had significant improvement in the HtSDS after treatment with rhGH (p: < 0.01), however, the BMISDS decreased significantly in the ISS group.

Table 4 compares the growth responses to rhGH therapy in GHD versus SGA groups. Both groups had significant improvement in the HtSDS after treatment with rhGH (p: < 0.01) with no significant change in their BMISDS.

Table 1. Anthropometric data of patients with short stature

		Age 1 (year)	Ht1 (cm)	Wt 1 (kg)	Age F (year)	HtF (cm)	Wt (kg)
SGA	Mean	8.20	111.80	19.90	11.00	130.50	28.1635
	SD	3.90	19.90	10.10	3.60	19.30	12.2661
TS	Mean	9.10	112.00	27.10	16.50	141.50	51.7
	SD	3.70	16.30	12.80	4.00	9.10	16.1
ISS	Mean	11.20	127.00	27.60	14.50	143.70	39.3
	SD	4.90	14.50	10.45	3.30	29.50	13.7
GHD	Mean	10.84	125.62	28.27	14.96	146.70	44.2
	SD	4.73	17.28	11.18	4.24	35.94	17.2

Legend: Small for Gestational Age (SGA); Turner Syndrome (TS); Idiopathic Short Stature (ISS); GH Deficiency (GHD); 1 = at start of GH therapy, F = at last examination.

Table 2. Growth data before and after long-term rhGH therapy in 4 groups of short children

			Age 1 (year)	HtSDS 1	BMISDS	Age 2 (year)	HtSDS 2	Age F (year)	HtSDS - F	BMISDS- F	Delta HtSDS	Delta BMISDS
GHD	n = 154	Mean	10.84	-3.84	-0.54	11.94	(-2.77) *	14.91	(-1.63) *	(-0.23) *	2.22	0.30
		SDS	4.73	1.90	1.55	4.08	1.12	4.27	1.22	2.14	1.33	2.14
SGA	n= 26	Mean	8.19	-2.43	-0.84	9.26	-1.91	11.01	(-0.96) *	(-1.94)*	1.46	-1.14
		SDS	3.98	2.26	1.71	3.68	1.45	3.57	1.21	2.06	1.36	1.75
TS	n= 9	Mean	9.11	-3.47	0.85	10.11	-2.95	16.51	-2.49	0.99	0.99	0.13
		SDS	3.69	1.38	1.16	3.69	1.13	4.02	1.09	0.98	1.44	0.45
ISS	n= 63	Mean	11.23	-1.94	-0.33	12.13	-1.73	14.54	-1.22	(-1.73)*	0.64	-1.32
		SDS	4.87	1.44	1.95	2.62	1.02	3.33	1.66	3.26	2.42	2.94
ANOVA		P-Value	0.001	<0.00001	0.054	0.001	<0.00001	0.00004	0.003	<0.00001	<0.00001	<0.00001

Legend: Small for Gestational Age (SGA); Turner syndrome (TS); idiopathic short stature (ISS); GH deficiency (GHD); *p: <0.05 after vs before rhGH therapy. ANOVA test among 4 groups. Age 1 = at first visit, Age 2 = after 1 year of therapy, Age F = age at the last visit. HtSDS: standing height in standard deviation; BMISDS:body mass index in standard deviation.

Table 3. Comparison between growth responses to rhGH therapy in GHD versus ISS groups

			Age 1 (year)	HtSDS 1	BMISDS	Age 2 (year)	HtSDS 2	Age F (year)	HtSDS - F	BMISDS - F	Delta HtSDS	Delta BMISDS
GHD	n = 154	mean	10.84	-3.84	-0.54	11.94	-2.77	14.91	-1.63	-0.23	2.22	0.30
		SDS	4.73	1.90		4.08	1.12	4.27	1.22	2.14	1.33	2.14
ISS	n= 63	mean	11.23	(-1.93)*	-0.33	12.13	(-1.72)*	14.54	-1.22	(-1.73)*	0.63*	(-1.32)*
		SDS	4.87	1.44	1.95	2.62	1.02	3.33	1.66	3.26	2.42	2.94

Legend: Idiopathic Short Stature (ISS); GH deficiency (GHD); *p<0.05 GHD vs ISS before and after GH therapy, Age 1 = at first visit, Age 2 = after 1 year of therapy, Age F = age at the last visit, t-test: 2 samples unequal variance. HtSDS: standing height in standard deviation; BMISDS:body mass index in standard deviation.

Table 4. Comparison between growth responses to rhGH therapy in GHD versus SGA groups

			Age 1 (year)	HtSDS 1	BMISDS	Age 2 (year)	HtSDS 2	Age F (year)	HtSDS - F	BMISDS - F	Delta HtSDS	Delta BMISDS
GHD	n = 154	Mean	10.84	-3.84	-0.54	11.94	-2.77	14.91	-1.63	-0.23	2.22	0.30
		SDS	4.73	1.90	0.40	4.08	1.12	4.27	1.22	2.14	1.33	2.14
SGA	n= 26	Mean	8.19*	(-2.42)*	-0.84	(9.25)*	(-1.91)*	(11.01)*	(-0.96)*	(-1.94)*	(1.45)*	(-1.13)*
		SDS	3.98	2.26	1.71	3.68	1.45	3.57	1.21	2.06	1.36	1.75

Legend: Idiopathic Short Stature (ISS); GH deficiency (GHD);* p<0.05 GHD vs SGA before and after rhGH therapy. Age 1 = at first visit, Age 2 = after 1 year of therapy, Age F = age at the last visit, t-test: 2 samples unequal variance. HtSDS: standing height in standard deviation; BMISDS: body mass index in standard deviation.

Table 5. Comparison between growth responses to rhGH therapy in GHD versus TS groups

			Age 1 (year)	HtSDS 1	BMISDS	Age 2 (year)	HtSDS 2	Age F (year)	HtSDS -F	BMISDS - F	Delta HtSDS	Delta BMISDS
GHD	n = 154	Mean	10.84	-3.84	-0.54	11.94	-2.77	14.91	-1.63	-0.23	2.22	0.30
		SDS	4.73	1.90	1.55	4.08	1.12	4.27	1.22	2.14	1.33	2.14
TS	n= 9	Mean	9.11	-3.47	0.85*	10.11	-2.95	16.51	-2.49	0.98*	0.98*	0.13
		SDS	3.69	1.38	1.16	3.69	1.13	4.02	1.09	0.97	1.44	0.45

Legend: GH deficiency (GHD); Turner syndrome (TS); * $p<0.05$ GHD vs SGA before and after GH therapy. Age 1 = at first visit, Age 2 = after 1 year of therapy, Age F = age at the last visit, t-test: 2 samples unequal variance. HtSDS: standing height in standard deviation; BMISDS: body mass index in standard deviation.

Table 6. Growth response to rhGH therapy in GHD and ISS during the first year of therapy and at the last visit; according to the duration of GH therapy

			Age 1 (year)	HtSDS 1	BMISDS	Age 2 (year)	HtSDS 2	Age F (year)	HtSDS - F	BMISDS -F	Delta HtSDS	Delta BMISDS
GHD												
>5 years	n = 45	mean	9.22	-4.48	-0.75	10.22	-3.05	17.23	-2.22	-0.61	2.26	0.14
		SDS	4.24	1.91	1.13	4.24	1.00	4.79	1.45	2.22	1.34	2.20
<5 years	n=110	mean	11.15	(-3.66)*	-0.45	12.15	(-2.69)*	13.81	(-1.42)*	0.24	2.24	0.68
		SDS	2.94	1.82	1.68	2.94	0.90	3.05	1.03	1.43	1.43	1.54
ISS												
>5 years	n = 15	mean	10.60	-2.09	-0.54	11.60	-1.83	17.51	-1.78	-1.59	0.31	-1.05
		SDS	3.48	1.50	2.07	3.48	0.99	3.67	0.98	2.46	0.90	1.85
<5 years	n= 49	mean	11.55	-1.89	-0.47	12.55	-1.70	14.02	-1.25	-1.38	0.64	-0.78
		SDS	2.42	1.51	1.95	2.42	1.10	2.48	1.80	2.40	0.52	1.93

Legend: Idiopathic Short Stature (ISS); GH deficiency (GHD); Age 1 = at first visit, Age 2 = after 1 year of therapy, Age F = age at the last visit, t-test: 2 samples unequal variance. HtSDS: standing height in standard deviation; BMISDS: body mass index in standard deviation.

The HtSDS improvement was significantly better in GHD children versus those with TS. On long-term rhGH therapy. The BMISDS did not differ significantly in children with GHD versus those with TS (Table 5).

The HtSDS changes improvement did not differ significantly between children with GHD or ISS treated for > 5 years with rhGH versus those treated for an average of 3 years (Table 6).

Discussion

From our analyses, the following results were obtained: (a) before rhGH treatment, the GHD group had a shorter HtSDS compared to those in the ISS and SGA groups; (b) the HtSDS increments on GH therapy were higher in the GHD group compared to those in ISS and SGA groups; (c) the improvement in the HtSDS of children with ISS occurred in the first 2-3 years of therapy and did not increase furtherly by increasing the duration of treatment; (d) the HtSDS in the GHD and ISS groups showed a significant increase up-to the end of the second year of treatment, and most children had HtSDS above -2 (normal) at the end of the third year of treatment (Table 2).

In support to our results, Lee et al. (28) reported that the effect of rhGH treatment on the final height in 25 children with GHD (11 organic and 14 idiopathic) was comparable to the their target heights. Similar results, showing an improvement of HtSDS from -4.13 to + 0.22, during 3.2 years of rhGH treatment with a dosage of 0.52-0.62 IU/kg/week was reported by Choi et al. (29) in 35 children with GHD (13 idiopathic and 22 organic).

Controversy still exists about the beneficial long-term effect of rhGH treatment in children with ISS. The growth-promoting effect of rhGH appears to be variable in these children. Our study demonstrates a significant but moderate improvement of HtSDS (0.65 SD) during rhGH therapy.

This improvement occurred during the first 2 years of treatment and was significantly lower compared to GHD patients. In support to our findings, Kim et al. (30) showed that the increase in the HtSDS in children with ISS was significantly lower compared to GHD, after 1 year of treatment using the same rhGH dose.

In another study, performed in children with familial short stature treated with rhGH therapy for more than 2 years at a dose of 0.23 mg/kg/week, the final HtSDS resulted not significantly different from the control group (31).

In 3 randomized controlled trials, the increment in adult height was 0.51 SDS (3.7 cm) with a rhGH dose of 0.22 mg/kg/week, for 4.4 years (32); 0.70 SDS (4.3-5.0 cm) with GH a dose of 0.23-0.47 mg/kg/week, for 5.9 years (24), and 1.23 SDS (7.5 cm) in female with a dose of 0.42 mg/kg/week, for 6.2 years (33).

In the current study, the dose of rhGH was 0.25-0.33 mg/kg/week and the height gain, after 3 years of therapy, was 0.65 SDS. On the other hand, Kang et al. (34) reported that HtSDS in ISS children improved initially with a rhGH dose of 0.23-0.35 mg/kg/week, but HtSDS did not significantly increase, after 2 years of treatment.

Sotos and Tokar (35) subdivided their children with ISS into familial and nonfamilial groups. A more favorable final height gain was reported in the non-familial ISS group. Similar findings were reported in a Korean study of children with familial short stature (36). We did not classify our ISS children into familial and non-familial ISS groups.

Park and Cohen (37,38), in order to increase maximally the growth response to rhGH proposed a dose regimen therapy with the aim to reach initially an IGF-1 target level of +2 to +3 SDS, followed by a lower IGF-1 level during the maintenance period. In our study, we did not adopt this model of treatment and a higher rhGH dose was not recommended for safety reasons. During rhGH therapy, we readjust the doses to maintain IGF-1 levels in the upper half of the normal range (from 0 to +2 SD) for their bone age.

Our ISS children received a higher rhGH dose than that given to GHD children. However, their HtSDS improvement was less evident when compared to the GHD group. During the treatment, two children complained of non-specific arthralgia that did not necessitate the discontinuation of treatment.

Growth retardation in infancy and short stature in childhood are associated with being born SGA. About 90% of children born SGA catch up to their genetic

height potential by about two years of age. Children born prematurely may take up to four years or more to catch up and are less likely to reach adequate stature than those born at term, especially if they were small for birth length.

Genetic predisposition, intrauterine programming, decreased GH secretion, reduced sensitivity to IGF-1, and GH resistance are suggested factors that may contribute to growth failure and short stature in children born SGA (39-42).

In the United States, rhGH is approved for the treatment of short SGA children whose height remains less than 2 SD below the mean for age and sex, at two years of age (43). In Europe, the approved indication is for short SGA children whose height is less than 2.5 SD below the mean for age and sex at four years of age (44).

Our prepubertal children with SGA, treated for about 3 years with rhGH (0.03-0.05mg/kg/day), increased their HtSDS by an average of 1.46 SD [22/26 (84.6%) of children attained HtSDS >-2 after treatment]. In support to our data, Van Pareren et al. (45) carried out a randomized, double-blind, dose-response study of long-term continuous rhGH therapy in short pre-pubertal SGA (birth length < -1.88 SD) children using the adult height as the end-point. Fifty-four children were treated with a rhGH dose of 0.23 or 0.47mg/kg/week, for an average of eight years, and compared, as a control group, the short pre-pubertal SGA children not treated with rhGH. The long-term continuous therapy of short SGA children resulted in normalization of height potential, during childhood and in adult final height, in most subjects compared with non-treated controls. Eighty-five percent of children treated with rhGH had final adult heights within the normal height range and 98% were within the target heigh range.

Our SGA children, treated for 3 years with rhGH, gained 1.45 SD in their stature. In support to our data, Dahlgren et al.(46) conducted a randomized control trial in 77 short pre-pubertal children born SGA (< -2 SD in birth length or birth weight) for over 8.5 years and compared them with 34 untreated short pre-pubertal SGA children. Long-term continuous rhGH therapy at a dose of 0.23 mg/kg/week resulted in an adult height close to height predicted by the parents' stature. The shortest, lightest, and youngest children had the best response to rhGH. Children receiving rhGH therapy for more than two years, prior to puberty, gained 1.7 SD in height (~12 cm in increased adult height) compared with those treated for less than two years prior puberty, who gained only 0.9 SD in height (~ 9 cm in increased adult height). Ninety percent of their children, treated with rhGH, achieved a final adult height within 1 SD of their target height compared with 50% of the untreated children born SGA. No adverse events drug-related were observed (46).

Carel et al. (47) performed a randomized controlled study with rhGH therapy in 102 SGA children (birth length < -2 SD) who presented with short stature around puberty age (mean age =12.7 years). The treated group received a dose of 0.47 mg/kg/week and was compared with 47 untreated short peripubertal SGA controls. rhGH therapy for 2.7 years performed during puberty significantly increased the final height of short SGA children compared with untreated short SGA children group. Forty-seven percent of the rhGH-treated children had final heights in the normal range compared to 27% of the control group. The authors observed that the height gain in the treated group was directly related to rhGH therapy duration and the earlier age at the beginning of therapy.

A meta-analysis reviewed long-term trials of 391 short SGA children treated with rhGH until adult height over the past decade. rhGH treatment dose ranged between 0.23-0.47 mg/kg/week. The mean height gain from the randomized control trials was 1.5 SDS (9.5 cm) in the rhGH treated children compared to 0.25 SDS (1.6 cm) of untreated children. The mean final adult height was -0.46 SDS in rhGH-treated SGA children compared to -1.26 SDS in untreated SGA children (48,49).

On the other hand, other authors believe that rhGH treatment is likely to yield only modest gains in height compared with no treatment (an increase in final adult height of approximately 6 cm, provided the treatment is begun early and continued for at least seven years) and concluded that their adult height was below average despite therapy (50).

Many studies have noted an association between intrauterine growth restriction and long-term health

risks, including type 2 diabetes, metabolic syndrome, and cardiovascular diseases. However, the mechanisms underlying this association have not been fully established.

Our observations, during treatment with rhGH of short children born SGA, have recognized the absence of safety issues as compared with other groups of children treated with rhGH. Only one patient developed symptoms of increased intracranial pressure (early morning headache) that disappeared after discontinuation of rhGH therapy. The parents refused to restart treatment.

Patients with TS should be treated with rhGH therapy to maximize their adult height and to improve body composition. In the United States, a typical initial dose of rhGH is 0.050 mg/kg/day (0.35 to 0.375 mg/kg/week), given once daily by subcutaneous injection. Patients with TS syndrome are typically treated with somewhat higher doses of rhGH compared to patients with GHD. Growth hormone therapy should be continued until little growth potential remains (e.g. bone age exceeds 13.5 to 14 years and growth slows to less than 2.5 cm per year) (51, 52).

Our TS patients who were treated with rhGH (0.033 to 0.066 mg/kg/day) for an average of 7 years had an improvement in HtSDS of 1 SD. However, 6/9 had HtSDS < -2 at the last examination.

Sas et al. (53) evaluated rhGH therapy in 68 young girls (mean age between 6 and 7 years) with TS who were randomly assigned to three different regimens of rhGH, starting at approximately 0.045 mg/kg/day, with some groups, and escalating the dose to approximately 0.090 mg/kg/day, during the first few years of therapy. They reported a normalization of height when the treatment was started at relatively young age and with higher doses of rhGH.

Another study, which followed 60 TS subjects on long-term rhGH therapy showed that 83 percent reached a normal adult height (54). Rising the rhGH dose, over time to around 0.075 mg/kg/day, they achieved a mean additional height gain of 5.3 cm. These height outcomes were achieved after an average treatment duration of 8.6 years. Similar results were reported from an observational registry of 344 patients with TS treated with rhGH (55). Effects of very early initiation of rhGH were evaluated in a prospective, randomized, open-label clinical trial in 88 girls with TS, aged 9 months to 4 years of age. rhGH was given for two years in a dose of 0.050 mg/kg/day to 45 girls versus no treatment in 43 TS girls. The treated group increased HtSDS by 1.6 ± 0.6 SD (p: <0.0001).

These studies confirm that early rhGH therapy can correct growth failure and normalize height potentials in toddlers with TS (56-59). A beneficial effect on bone growth and body composition with increased lean body mass and decreased body fat has been also reported (57-59). In our patients with TS, the BMIS-DS did not show any significant change during rhGH therapy. Furthermore, rhGH treatment does not appear to have a deleterious effect on blood pressure, left ventricular function, or aortic diameter. None of our TS patients had any of the side effects related to rhGH therapy, including slipped capital femoral epiphyses, intracranial hypertension, and pancreatitis (60).

In conclusion, children with SGA, ISS and TS groups showed significant increases in HtSDS when treated with rhGH for 2 to 3 years. However, their HtSDS increments were significantly lower than those attained in children with GHD. No deleterious side effects requiring cessation of therapy were registered in the majority of our children.

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Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Preliminary experience in the treatment of hip necrosis with BIOS screws associated with growth factors

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Summary. *Introduction:* In this paper we present the preliminary results obtained in our clinic with the use of BIOS screws associated with injection of growth factors in the treatment of cephalic necrosis of the femoral head. *Materials and methods:* In the division of Orthopedics and Traumatology at Guglielmo da Saliceto" hospital in Piacenza were treated between 2012 and 2016 with the proposed technique 8 case of necrosis of the femoral head in 6 patients with a mean age of 41.8 years (between 31 and 60 years). All patients before surgery were affected by debilitating pain with VAS greater than 7 and functional limitation of the range of motion of the hip. In all patients was performed a decompression of the femoral head using cannulated screws BIOS and injection through the implants of growth factors. *Results:* Our results have been extremely positive. All patients treated at two months have reported the resolution of the pain that affected them before surgery and the recovery of the function of the hip. At the last clinical control carried out no patient experienced recurrence of any symptoms related to necrosis. *Discussion:* Cephalic necrosis due to various possible etiologies is a not so rare condition, often affecting young patients with high functional demands. Only few years ago the gold standard treatment for this kind of pathological condition was hip prosthesis and this fact was associated with long term complications related with the young age of patients. The treatment presented is simple, fast and very effective in allowing the penetration of growth factors through bony trabeculae of the femoral head, due to the fenestratures inside the screws. The results obtained in our experience are certainly promising, though longer follow-up is needed to evaluate the functional outcome long and to evaluate the possible re-emergence of pain. *Conclusion:* Conservative treatment using BIOS screws associated with growth factors in femoral head necrosis in our experience combine a low invasiveness to excellent functional results and should therefore be considered a valid option in treating this pathology in young patients. (www.actabiomedica.it)

Key words: BIOS screws, growth factors, cephalic necrosis, hip surgery, conservative surgery

Introduction

Necrosis of the femoral head is a not so rare pathology that often affect relatively young patient, providing important functional limitations and pain in active people with high functional request (1).

The most used classification was the one proposed by Ficat that, in the modified version elaborated in 1985, recognize 5 stages in the progression of the necrosis (2).

In this work we present our preliminary experience in the conservative treatment with BIOS screws associated with the use of growth factors.

This technique is quite interesting, associating the decompression of the femoral head with a deep penetration of the growth factors in the bone.

Materials and methods

In the division of Orthopedics and Traumatology at "Guglielmo da Saliceto" hospital in Piacenza between 2012 and 2016 we treated 8 case of idiopatic necrosis of the femoral head in 6 patients with a mean age of 41.8 years (between 31 and 60 years).

4 patients were male(including the 2 bilateral) and 2 were female.

All patients before surgery were affected by debilitating pain with VAS greater than 7 and functional limitation of the range of motion of the hip.

In all patients was performed a decompression of the femoral head using a single cannulated screws BIOS associated with a perforation just superior to this one and injection through the implants of growth factors. The screw are provided with internal holes that consent the penetration of the growth factors directly in the bony trabeculae.

We performed the complete procedure in about 15 minutes in all cases.

All the patients were evaluated clinically and with x rays at 1, 3, 6 and 12 months and at last clinical control.

The results were valuated considering VAS,HHS and major complications.

Average follow up was of 27 months (from 12 to 54 months).

Results

The results obtained, although the casuistry is still small and the follow-up must be prolonged, has been very positive.

At the final clinical control available all the patients were satisfied about the procedure and none of them referred a significant pain or limitation in the range of motion of the affected hip.

The average final Harris hip score was 89 and no patient developed any relevant complication. Average VAS was 1,4 (from 1 to 3) with any patient complaining for pain during the ADL.

Discussion

Cephalic necrosis is a not so rare pathological condition with heavy consequences on the affected patients,due to their young age and high functional request.

In the last years this has been one of the field of the orthopaedic science in which the use of the new growth factors has found an important application.

In literature we find a lot of paper reporting interesting results with the use of these growth factors (3) both in animal models representing a similar condition (4) and in the clinical practice, mostly associated with a core decompression (5, 6).

The screws we used may be considered an evolution of the treatment realized by core decompression, adding to the advantages offered by this procedure the chance to offer a mechanic support and to distribute uniformly the pressure when inserting the growth factor on a wider surface, consenting a large penetration in the trabecular bone, without increasing the requested amount of time for surgery or invasiveness for the patients.

Conclusion

The conservative treatment with BIOS screws in our preliminar experience seems a valuable option for the treatment of femoral head necrosis of the I and II type according to Ficat.

This procedure combining the mechanic effect similar to the one offered by the classical procedure of core decompression with the use of growth factors directly in the bone affected by the pathological condition seems very promising, although a longer follow up and a larger casuistry must be analyzed to obtain statistically significant data.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Gas gangrene, diabetes and amputations of upper extremities

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Summary. *Purposes:* The aim of the current study was to evaluate epidemiological data on amputations of upper extremities. *Methods:* The main causes of upper extremity amputations performed in the period from January 1998 to January 2008 in Hospital de Base, São José do Rio Preto were retrospectively evaluated in a descriptive and quantitative cross-sectional study. Data, including the age of the patient, gender and the reason for surgery, were obtained from hospital records identified by the international classification of diseases (ICD) code for amputation. *Results:* A total of 2919 amputations were performed in the period of this study with only 23 involving the upper extremities; thus 22 patients were included in this study as one was submitted to amputation of both arms. Fifteen patients (65.21%) were male with ages that ranged between 18 and 84 years old (mean = 41.6 years old). Seven patients (34.79%) were women with ages from 24 to 87 years old (mean = 58.8 years old). The causes for amputation were: accidents (14), gas gangrene (4), malignant neoplasms (3), arterial thrombosis (1) and unidentified cause (1). *Conclusion:* Gas gangrene of the upper extremities is associated to diabetes mellitus which highlights the severity of the disease. (www.actabiomedica.it)

Key words: gas gangrene, diabetes, amputations, upper extremities

Introduction

Upper extremity amputations are not as common as lower extremity amputations, but they present unique challenges to the surgeon, prosthetist and amputee (1). The predictors of amputation for patients with lower extremity vascular trauma are well described in the literature, but the predictors of amputation of upper extremities are not so well defined. One study suggests that for the vast majority of upper extremity injuries, salvage should have been attempted regardless of the severity score (2).

The main causes of amputation of upper extremities are accidents, neoplasms, ischemia and infection (1-5).

Although upper extremity ischemia is rare, results for upper extremity bypass are excellent, superior to those reported for lower extremity ischemia (3). Gas gangrene of the upper limb is rare, acutely painful and rapidly fatal (4). The primary goal of treatment for malignant bone and soft tissue tumors of the hand and upper extremity is an oncological cure. Despite recent advances in chemotherapy and radiation techniques, amputation still provides a means to achieve surgical cure for some bone and soft tissue sarcomas (5). Patients submitted to major amputations of the upper extremity show high psychological and functional impairment. Pain, deficits in function and still non-optimal prosthetic devices result in a high percentage of unemployment (6).

The aim of the current study was to evaluate epidemiological data of amputations of the upper extremities in a teaching hospital.

Methods

The main causes of amputations of upper extremities performed in the period from January 1998

to January 2008 in Hospital de Base, São José do Rio Preto were retrospectively evaluated in a descriptive and quantitative cross-sectional study.

Data, including the age of the patient, gender and the reason for surgery were obtained from hospital records identified by the international classification of diseases (ICD) code for amputation. The data were input on an Excel spreadsheet with the frequency of the events being used for statistical analysis.

This work was approved by the Research Ethics Committee of the Medicine School in São José do Rio Preto (FAMERP)-Brazil.

Results

Of a total of 2919 amputations performed in the study period, only 23 procedures involved the upper extremities. In total only 22 patients were included in this study as one individual was submitted to amputation of both upper extremities. Fifteen patients (65.21%) were male with ages that ranged between 18 and 84 years old (mean = 41.6 years). Seven patients (34.79%) were women with ages from 24 to 87 years old (mean = 58.8 years).

The causes of amputation were: accidents (14), gas gangrene (4), malignant neoplasms (3), arterial thrombosis (1) and unidentified cause (1). Of the 14 trauma patients, the indication of amputation was due to vascular lesions. Of the 4 patients submitted to amputations for gas gangrene, 3 were diabetics.

Discussion

This study found that trauma, gas gangrene and neoplasms were the three main causes of amputations of the upper extremities and warns about the association between diabetes and amputations due to gas gangrene. In the literature there are only a few case reports that mention gas gangrene of the upper extremities (6-8). In respect to the lower extremities, there is a correlation between infection by gas gangrene and diabetes (9). Another aspect that should be considered is the death rate of individuals with gas gangrene of the lower extremities at around 30% of the cases (9). For the upper extremities, there was one death in four cases with gas gangrene. Thus, this serves as a warning about the seriousness of gas gangrene associated with diabetes and the possibility of death.

Amputations of lower extremities are more common than of upper extremities. In respect to age, female patients submitted to amputations of the arms were older than male patients. This difference may be explained by the fact that men suffer more accidents than women in this region (10).

Injuries of arms have consequences that limit all the aspects of human activities. In rehabilitation, the movements of upper extremities are more specific and precise than movements of the lower extremities and thus greater functional limitations are experienced. Self-reported upper extremity health status and quality of life following amputation of the hand can be in contrast to the objective pathology. In patient-oriented assessment of results, individual psychosocial factors that may affect results must be taken into consideration (11).

An upper extremity disability should be evaluated after the completion of treatment and full adaptation when further functional changes are not expected. The dominance of the right or left hand before the disability should not be considered when there is a high rate of disability (12).

Another important aspect in respect to trauma is the treatment provided. Delay in surgery, blunt trauma and extensive soft tissue defects in combined orthopedic and vascular injuries were associated with increased risk of amputation, while associated nerve injuries and bone injuries with extensive soft tissue damage are risk factors of a poor quality outcome (13).

Conclusion

Gas gangrene of the upper extremities is associated with diabetes mellitus and serves as a warning in respect to the severity of the patient's disease.

Authors' Contributions: All authors participated and contributed to all phases of the study

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Biochemical, immunochemical and serology analytes validation of the lithium heparin BD Barricor blood collection tube on a highly automated Roche COBAS8000 instrument

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Summary. *Background:* Recently developed blood tubes with a barrier to provide plasma are becoming widespread. We compared 43 biochemical, 35 immunochemical and 7 serology analytes in a BD-Vacutainer® Barricor tube for local clinical validation of this lithium-heparin tube with a barrier. *Methods:* Samples from 70 volunteers were collected in different BD-tubes: a clot-activator tube with gel (SST), a lithium-heparin tube with gel (PST), and a lithium-heparin tube with barrier (BAR). Biases from Bland-Altman plots and 95% confidence intervals were compared with the desirable specification from the Ricos database in order to verify whether measurements from different tubes were significantly different. *Results:* For most of the analytes tested, the measurements using SST, PST or BAR tubes were equivalent. Only BIC, GLU, K, LAD, LPA, P, TP, CTX, Ferritin, HGH, vitD3 and ANTIS showed statistically significant, between-tubes, differences which might have clinical implication. *Conclusions:* The study demonstrates that SST, PST and BAR can be used interchangeably for most of the analytes tested, including serology analytes. This allows the use of the same tube for assaying multiple analytes, increasing the laboratory efficiency while decreasing patients discomfort by minimizing blood withdrawal. (www.actabiomedica.it)

Key words: blood collection tube, mechanical separator, plasma, serum, serology

1. Introduction

The preanalytical phase plays a crucial role in laboratory diagnostic and blood collection is probably its most important aspect (1). Heparin plasma and serum are commonly used matrices. The latter is the preferred specimen for the analysis of biochemical parameters (2,3), nevertheless plasma has some important laboratory advantages like a shorter turnaround time (TAT) due to both the absence of the 30-60 minutes time interval needed for the coagulation process (4) and to a shorter centrifugation step, and allows to obtain a larger volume of sample (about 15-20% more) which increases the number of analysis that can be made on one sample (5). According to the World Health Organization, plasma is preferred to serum because it reflects better the patients' physiological condition (6) by preventing the changes induced by the coagulation process which causes an increase in some analytes (e.g. potassium) and a decrease of others (e.g. total proteins) (7). In addition, the use of anticoagulant prevents the variations induced by the coagulation factors activated when the needle is inserted. The use of plasma also minimizes the formation of fibrin networks found

very frequently in serum tubes for several reasons: the sample arrived quickly in the laboratory (e.g. through pneumatic mail systems) and is centrifuged before clot formation, or because the sample was from patients taking oral anticoagulants or heparin which delayed the formation of the clot (8). The presence in serum of soluble fibrin clots causes, on highly automated analytical lines, frequent sampling alarms requiring re-centrifugation or manual re-run of the sample leading to a large increase of the TAT.

Blood collection tubes (BCT) with a gel separator are often the preferred choice because serum (plasma) is physically separated from clotted whole blood (blood cells) (3). However, some drawbacks may still occur like the non-specific adsorption of the molecule to be analyzed or the release of interfering substances (9). A new BCT, the BD-Barricor tube (BAR), containing lithium heparin as anticoagulant and an innovative mechanical separator has been recently developed. According to the manufacturer BAR will improve the quality of laboratory routine analysis in term of TAT and analytes stability. A few studies comparing BAR with standard plasma or serum tubes have been published (10-13) but they still do not cover the wide range of analytes tested in routine analysis. Furthermore, to the best of our knowledge, the BD-Barricor tube has never been tested before for serological analytes. Given this lack of data, 43 biochemical analytes, 35 immunochemical analytes and 7 serology analytes were tested on a fully automated Roche COBAS8000 instrumentation. The study aimed at verify whether plasma (either standard or BAR tubes) can replace serum for high throughput routine analysis without affecting the normal clinical ranges suggested by the manufacturer or selected by the Laboratory.

2. Materials and methods

2.1. Subjects and blood sampling

A total of 70 apparently healthy volunteers, 29 males and 41 females from the San Raffaele Hospital in Milan, Italy were included in the study during the period April-June 2017. Volunteers were aged between 18 and 70 and had no pregnancy status. During blood collection from the volunteers, no exclusion criteria were applied with the exception of difficulties in blood withdrawal like inability to find a suitable vein. Blood samples were collected after overnight fasting (8-10 hours), between the hours of 08:00 and 10:00 am. Smoking and the consumption of tea or coffee were forbidden from midnight until blood collection. Alcohol consumption was not allowed for 3 days prior to blood sampling. Volunteers were seated in an upright position 1 minute before venipuncture and remained seated during the whole procedure. Blood samples were collected, as described elsewhere (14, 15), into three different BCTs from BD (Becton, Dickinson and Company, NJ): a clot-activator gel-containing tube BD-SST II Advance tube, 3.5 mL, 13x75 mm (SST); a lithium heparinized gel-containing tube BD-PST II, 3.0 mL, 13x75 mm (PST); a lithium heparinized tube with a barrier BD-Barricor, 3 ml, 13x75 mm (BAR). PST and BAR were processed immediately after sample collection whereas SST was incubated for at least 30' to allow appropriate clotting. Samples were separated by centrifugation at 3000xg for 10' at 4°C. No visible hemolysis was detected in any sample. Concentration measurements were performed within 4 hours after blood collection.

A total of 85 parameters were measured on a Roche COBAS 8000 device (Roche Diagnostic, Basel, Switzerland). Among them 43 were routine biochemical analytes including albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), amylase (AMS), pancreatic amylase (AMSP), antistreptolysin O (ASO), aspartate aminotransferase (AST), beta-2 microglobulin (B2MICR), bicarbonate (BIC), total bilirubin (BILT), complement C3 (C3), complement C4 (C4), calcium (Ca), cholinesterase (CHE), creatine kinase (CK), chloride (CL), cholesterol (CHO), creatinine (CREA), C-reactive protein (CRP), iron (Fe), gamma-glutamyl transferase (GGT), glucose (GLU), high-density lipoprotein (HDL), homocysteine (HOM), immunoglobulin A (IGA), immunoglobulin G (IGG), immunoglobulin M (IGM), potassium (K), lactate dehydrogenase (LAD), lipase (LIP), lipoprotein A (LPA), low-density lipoprotein (LDL), magnesium (MG), mucoproteins (MUCO), sodium (NA), procalcitonin (PCT), phosphate (P), rheumatoid factor (RF), total protein (TP), transfer-

rin (TRF), triglyceride (TG), urea (UREA), uric acid (UA). The 35 routine immunochemical analytes include: alpha-fetoprotein (AFP), vitamin B12 (B12), beta human chorionic gonadotropin (BHCG), cancer antigen 125 (CA125), cancer antigen 15-3 (CA153), carcinoembryonic antigen (CEA), creatine kinase-muscle/brain (CKMB), cortisol (CORT), peptide-C (CPEP), calcitonin (CA), serum C-telopeptide (CTX), estradiol (E2), ferritin, folate, follicle-stimulating hormone (FSH), free triiodothyronine (FT3), free thyroxine (FT4), cancer antigen 19-9 (GICA), osteocalcin (GLA), growth hormone (HGH), human prolactin (HPRL), insulin (INS), Luteinizing hormone (LH), myoglobin (MIOG), N-terminal-pro BNP (PROBNP), progesterone (PROG), total prostate-specific antigen (PSA), triiodothyronine (T3), thyroxine (T4), testosterone (TESTO), thyroglobulin antibodies (TGAB), anti-thyroid peroxidase (TPO), high-sensitivity cardiac troponin T (hs-CTnT), thyroid stimulating hormone (TSH), and vitamin D3 (vitD3). A total of 7 routine serology analytes were also measured: anti-hepatitis B antibodies (ANTIS), Cytomegalovirus IgG antibodies (CMVG), Cytomegalovirus IgM antibodies (CMVM), Rubella IgG antibodies (RUBEOG), Rubella IgM antibodies (RUBEOM), Toxoplasma gondii IgG antibodies (TOXOG) and Toxoplasma gondii IgM antibodies (TOXOM).

Table 1 shows a brief description of the method used for each analyte.

Individuals signed an informed consent authorizing the use of their anonymously collected data for retrospective observational studies (article 9.2.j; EU general data protection regulation 2016/679 [GDPR]), according to the San Raffaele Hospital policy (IOG075/2016).

2.2. Statistical analyses

Statistical analyses and graphs were performed with the software Sigmaplot (Systat-Software, Inc. San Jose, CA, USA) and Excel (Microsoft, Redmond, WA, USA). Comparisons between SST, PST and BAR were assessed by the Bland-Altman (BA) plot (17). To avoid disproportionate weights due to analytes having wide concentration ranges the calculated mean bias, and the corresponding 95% confidence interval (95%CI) were expressed as percentage. The latter was compared with the desirable specification (B%) obtained from the Ricos database (18). ASO, MUCO, PCT, UA, BHCG, CT, vitD3 and serological analytes, for which B% was not available, were compared with a 5% arbitrary threshold. The 95CI% was calculated as: bias ± t(0.025; df=n-1)SE, where bias is the calculated % mean bias, SE the standard error of the n differences, with t from the t distribution with n-1 degrees of freedom. The mean %bias was considered statistically significant if its calculated 95%CI did not included the zero; if the 95%CI also exceeded the B%, the mean %bias was considered clinically significant. However, if the 95CI% exceeded the B% but contained also the zero, we cautiously preferred not to make any statement.

3. Results

Collected blood was first tested for hemolysis by measuring the free hemoglobin (fHb), using the hemolysis index (HI). The Roche instrumentation estimates the HI by dichromatic wavelength paired measurement, providing results as absolute numbers, where one unit corresponds to 0.01 g/L. PST and BAR tubes showed fHb of 0.045 and 0.040 g/L, respectively whereas the SST tubes exhibit slightly higher hemolysis (0.070 g/L). However, after a one way ANOVA test, only SST and BAR showed a statistically significant difference.

Table 2-4 show the summary of the BA comparisons between the three BCTs and the corresponding B%.

3.1. Biochemical analytes

BIC, K, LAD, LPA, P, and TP showed 95CI% clinically significant only when serum was compared to plasma. Within the same analyte, the 95CI% were similar regardless of the type of plasma tube used. In contrast Ca (which was associated to a rather small B%) and GLU, showed significantly different 95CI% in all of the three comparisons. Na and TRF, also associated to small B%, showed significantly different

Table 1. List of the analytes measured in this study and their corresponding methodology

Biochemical		**Immunochemical**	
Analyte	**Method**	**Analyte**	**Method**
ALB	Immunoturbidimetric assay	AFP	electrochemiluminescence
ALP	Colorimetric assay	B12	electrochemiluminescence
ALT	Spettrophotometric assay	BHCG	electrochemiluminescence
AMS	Enzymatic-colorimetric	CA125	electrochemiluminescence
AMSP	Enzymatic-colorimetric	CA153	electrochemiluminescence
ASO	Immunoturbidimetric assay	CEA	electrochemiluminescence
AST	Spettrophotometric assay	CKMB	electrochemiluminescence
B2MICR	Immunoturbidimetric assay	CORT	electrochemiluminescence
BIC	Enzymatic assay	CPEP	electrochemiluminescence
BILT	Colorimetric assay	CT	electrochemiluminescence
C3	Immunoturbidimetric assay	CTX	electrochemiluminescence
C4	Immunoturbidimetric assay	E2	electrochemiluminescence
Ca	Colorimetric assay	Ferritin	electrochemiluminescence
CHE	Colorimetric assay	Folate	electrochemiluminescence
CK	Spettrophotometric assay	FSH	electrochemiluminescence
CL	Potentiometric assay	FT3	electrochemiluminescence
CHO	Enzymatic/colorimetric assay	FT4	electrochemiluminescence
CREA	Colorimetric assay	GICA	electrochemiluminescence
CRP	Immunoturbidimetric assay	GLA	electrochemiluminescence
FE	Colorimetric assay	HGH	electrochemiluminescence
GGT	Enzymatic/colorimetric assay	HPRL	electrochemiluminescence
GLU	Enzymatic assay	HS-CTnT	electrochemiluminescence
HDL	Enzymatic/colorimetric assay	INS	electrochemiluminescence
HOM	Enzymatic assay	LH	electrochemiluminescence
IGA	Immunoturbidimetric assay	MIOG	electrochemiluminescence
IGG	Immunoturbidimetric assay	PROPNB	electrochemiluminescence
IGM	Immunoturbidimetric assay	PROG	electrochemiluminescence
K	Potentiometric assay	PSA	electrochemiluminescence
LAD	Spettrophotometric assay	T3	electrochemiluminescence
LIP	Enzymatic/colorimetric assay	T4	electrochemiluminescence
LPA	Turbidimetric assay	TESTO	electrochemiluminescence
LDL	Enzymatic/colorimetric assay	TGAB	electrochemiluminescence
MG	Colorimetric assay	TPO	electrochemiluminescence
MUCO	Immunoturbidimetric assay	TSH	electrochemiluminescence
NA	Potentiometric assay	VitD3	electrochemiluminescence
PCT	Immunoturbidimetric assay	**Serology**	
P	Spettrophotometric assay	ANTIS	electrochemiluminescence
RF	Immunoturbidimetric assay	CMVG	electrochemiluminescence
TP	Colorimetric assay	CMVM	electrochemiluminescence
TRF	Immunoturbidimetric assay	RUBEOG	electrochemiluminescence
TG	Enzymatic/colorimetric assay	RUBEOM	electrochemiluminescence
UREA	Enzymatic assay	TOXOG	electrochemiluminescence
UA	Enzymatic/colorimetric assay	TOXOM	electrochemiluminescence

Table 2. Biochemical analytes. For each comparison is shown the number of tests (n), the BA Bias calculated as percentage (%bias), the confidence interval (95CI%) and the biological variation expressed as desirable specification for inaccuracy (B%) (18). Analytes with a 95CI% exceeding B% are highlighted in grey

Analyte	n	SST vs Barricor		SST vs PST		Barricor vs PST		B%
		%bias	95CI%	%bias	95CI%	%bias	95CI%	
ALB	69	0.9	0.32, 1.39	0.8	0.16, 1.37	-0.2	-0.75, 0.37	1.4
ALP	69	2.0	1.39, 2.92	2.7	2.41, 3.67	0.7	0.16, 1.63	6.7
ALT	70	3.9	1.39, 5.96	-1.4	-3.80, 0.64	-5.3	-7.52, -2.97	11.5
AMS	70	-0.7	-1.20, 0.22	-0.4	-0.91, 0.00	0.3	-0.24, 0.77	7.4
AMSP	70	-0.5	-1.34, 0.25	0.0	-0.74, 0.74	-0.5	-0.04, 1.12	8.0
ASO	70	-2.0	-4.51, 0.61	-0.9	-3.30, 1.48	1.1	-1.48, 3.30	5*
AST	62	1.2	-1.61, 4.02	-1.2	-4.52, 1.98	-2.4	-0.27, 5.14	6.5
B2MICR	66	0.6	0.07, 1.22	0.7	0.16, 1.33	0.2	-0.29, 0.62	4.1
BIC	68	-5.9	-7.79, -3.77	-5.9	-7.91, -4.00	-0.3	-1.78, 1.19	1.6
BILT	69	-0.6	-1.91, 0.79	-0.7	-1.98, 0.85	-0.2	-1.64, 1.29	8.9
C3	70	-3.1	-4.04, 0,75	-0.3	-1.13, 0.37	2.8	0.45, 3.58	4.1
C4	68	0.0	-0.81, 0.74	0.8	0.06, 1.49	0.8	0.02, 1.47	8.6
Ca	69	0.8	0.08, 1.73	-0.8	-1.58, -0.25	-1.8	-2.51, -1.10	0.8
CHE	70	0.5	-0.08, 1.18	0.8	0.21, 1.40	0.3	-0.28, 0.81	4.8
CK	69	2.2	1.19, 3.19	1.6	0.70, 2.39	-0.6	-1.62, 0.34	11.5
CL	70	0.3	-0.14, 0.46	-0.1	-0.38, 0.25	-0.4	-0.48, -0,08	0.5
CHO	69	0.1	-0.43, 0.72	1.0	0.37, 1.68	0.8	0.22, 1.45	4.1
CREA	70	-1.5	-2.45, -0.50	-1.0	-1.81, -0.03	0.5	-0.49, 1.61	4.0
CRP	70	-2.1	-3.87, -0.13	-0.3	-1.47, 0.96	1.7	-3.98, 0.48	21.8
FE	69	1.3	0.50, 2.00	1.6	0.85, 2.19	0.3	-0.10, 0.75	8.8
GGT	70	2.2	0.53, 5.13	0.9	-0.87, 2.50	-1.3	-0.37, 4.41	11.1
GLU	70	3.9	2.42, 5.45	2.3	0.53, 4.20	-1.6	-2.72, -0.47	2.3
HDL	70	-0.1	-0.74, 0.59	0.0	-0.43, 0.47	0.1	-0.71, 0.47	5.6
HOM	63	0.1	-0.94, 1.17	-0.9	-2.07, 0.23	-1.2	0.23, 2.09	8.6
IGA	70	-0.2	-1.08, 0.70	0.5	-0.67, 1.65	0.6	-1.59, 0.29	9.1
IGG	69	0.5	0.06, 0.95	0.7	0.23, 1.13	0.1	-0.62, 0.32	4.3
IGM	69	0.4	-0.12, 1.02	1.6	0.67, 2.47	1.1	-2.01, -0.24	11.9
K	70	7.6	6.47, 8.62	7.2	6.25, 8.23	-0.4	-1.21, 0.46	1.8
LAD	70	-3.9	-6.09, -1.40	-3.9	-6.24, -1.24	0.1	-2.38, 2.39	4.3
LIP	68	0.1	-0.52, 0.69	0.3	-0.37, 0.84	0.1	-0.67, 0.53	11.3
LPA	54	-4.7	-8.24, -1.22	-4.4	-7.70, -1.03	-0.3	-1.87, 2.48	3.7
LDL	68	0.3	-0.19, 0.88	0.6	0.06, 1.19	0.3	-0.83, 0.23	5.5
MG	69	0.1	-0.61, 0.82	0.3	-0.31, 0.92	0.2	-0.83, 0.40	1.8
MUCO	70	0.2	-0.87, 0.90	0.9	0.09, 1.61	0.6	-1.45, 0.23	5*
NA	69	0.0	-0.19, 0.19	0.2	0.01, 0.39	0.2	-0.47, -0.09	0.2
PCT	64	-4.9	-19.19, 7.69	-34.3	-50.87, -17.69	-33.5	-15.51, -51.39	5*
P	70	7.6	6.67, 8.31	5.0	3.96, 6.05	-2.6	-1.42, -3.28	3.4
RF	69	0.5	-0.02, 0.66	0.7	-0.01, 1.27	0.3	-0.15, 0.66	6.5
TP	69	-4.5	-5.20, -3.70	-4.1	-4.95, -3.25	-0.4	-0.98, 0.25	1.4
TRF	68	0.1	-0.70, 0.98	1.3	0.42, 2.04	-1.2	-1.91, -0.41	1.3
TG	70	0.8	-0.01, 1.56	3.5	2.59, 4.40	2.7	3.46, 1.90	9.6
UREA	70	-1.5	-2.49, -0.57	-0.5	-1.41, 0.26	1.0	-1.76, 0.01	5.6
UA	70	0.3	-0.58, 1.10	0.0	-0.69, 0.57	-0.3	-0.93, 0.50	5*

Table 3. Immunochemical analytes. For each comparison was shown: the number of tests (n), the BA Bias calculated as percentage (%bias), the confidence interval (95CI%) and the biological variation expressed as desirable specification for inaccuracy (B%) (18). Analytes with a 95CI% exceeding B% are highlighted in grey.

Analyte	n	SST vs Barricor		SST vs PST		Barricor vs PST		B%
		%bias	95CI%	%bias	95CI%	%bias	95CI%	
AFP	63	0.4	-0.39, 1.20	0.2	-0.89, 1.28	-0.2	-1.25, 0.80	11.8
B12	68	0.9	-0.31, 2.11	1.5	0.31, 2.66	0.8	-0.23, 1.84	17.7
BHCG	70	-1.2	-3.04, 0.61	-0.7	-2.27, 0.79	0.5	-0.50, 1.46	5*
CA125	65	1.6	0.88, 2.37	2.4	1.68, 3.06	0.7	-0.11, 1.60	15.0
CA153	65	1.7	-0.06, 3.43	2.7	0.88, 4.46	1.0	-0.80, 2.78	15.8
CEA	65	2.0	1.03, 2.93	2.5	1.42, 3.47	0.5	-0.64, 1.58	14.3
CKMB	64	2.3	-0.09, 4.73	1.5	-2.3, 3.49	0.1	-2.05, 2.26	7.8
CORT	64	-1.1	-4.02, 1.74	1.2	-1.89, 4.35	2.1	-0.93, 5.19	7.6
CPEP	64	-1.3	-3.52, 0.98	-0.9	-2.83, 1.05	0.4	-1.47, 2.22	7.1
CT	65	-0.4	-2.09, 1.30	-3.8	-4.91, 1.66	-3.5	-4.98, -1.50	5*
CTX	64	-8.3	-9.92, -6.40	-5.2	-6.92, -3.46	3.1	1.77, 4.41	8.1
E2	68	-2.4	-12.2, 7.49	8.7	-1.99, 19.32	9.1	-0.68, 18.6	8.3
Ferritin	70	-3.9	-6.80, -1.18	-0.2	-2.11, 1.31	3.7	1.04, 6.17	5.2
Folate	69	-3.2	-6.36, 0.04	-3.7	-7.19, 0.05	-0.5	-3.27, 2.25	19.2
FSH	66	1.0	0.60, 1.40	0.9	0.35, 1.35	-0.1	-0.58, 0.32	12.1
FT3	68	-0.4	-2.51, 1.78	-0.6	-2.74, 1.48	-0.3	-1.58, 2.12	4.8
FT4	68	0.5	-0.10, 1.01	0.7	0.25, 1.29	0.3	-0.19, 0.83	3.3
GICA	64	0.4	-0.15, 0.91	0.6	-0.02, 1.12	0.2	-0.81, 0.32	32.9
GLA	57	-1.6	-3.34, 0.21	-1.3	-2.96, 1.29	0.3	-1.94, 1.31	7.9
HGH	64	13.4	4.38, 21.18	12.9	4.01, 20.94	-0.3	-0.31, 0.96	12.2
HPRL	62	-1.1	-1.74, -0.51	-0.7	-1.03, 0.01	0.3	-1.02, 0.38	10.5
HS-CTnT	70	-0.6	-4.27, 3.01	-3.1	-5.17, 0.06	-2.4	-0.73, 5.67	7.0
INS	65	2.9	-0.71, 6.53	5.4	2.65, 8.15	2.5	-5.08, 0.10	15.5
LH	64	-3.8	-4.55, 2.97	-3.5	-4.23, -2.85	-0.2	-0.71, 0.36	8.9
MIOG	64	-3.3	-5.44, -1.18	-2.9	-4.44, -1.43	0.4	-2.25, 0.80	8.2
PROBNP	66	0.2	-2.01, 2.33	0.6	-1.41, 2.59	0.5	-1.87, 2.93	4.7
PROG	66	-10.3	-19.39, 0.25	-2.0	-14.83, 10.90	6.9	-16.47, 2.63	13.5
PSA	26	0.1	-2.23, 2.30	-0.7	-3.19, 1.83	-0.	-1.04, 2.48	18.7
T3	64	0.6	-0.25, 1.39	0.4	-0.50, 1.13	-0.3	-0.52, 1.02	5.2
T4	65	1.7	1.16, 2.30	2.4	1.86, 2.92	0.7	-1.21, -0.10	3.0
TESTO	28	-1.7	-3.78, 0.34	0.6	-1.91, 3.13	2.3	-5.31, 0.66	6.0
TGAB	63	-32.8	-39.40, -26.26	-28.2	-34.54, -21,85	5.3	-10.21, 0.23	20.6
TPO	61	-2.5	-17.95, 13.03	-18.5	-31.25, 0.28	-16.6	-0.65, 25.44	5.7
TSH	68	2.2	1.23, 3.22	-0.2	-1.12, 0.64	-2.5	1.54, 3.39	9.7
vitD3	65	1.2	-0.65, 3.75	-3.2	-5.30, -0.94	-4.4	-6.49, -2.39	5*

95CI% only when PPT was used whereas SST and BAR were equivalent.

PCT showed 95CI% significantly different from the arbitrary adopted B% when PPT was used, however, the 95CI% amplitudes were very large. In contrast, when SST was compared to BAR the 95CI% became almost ten time smaller, but still exceeded the B%.

3.2. Immunochemical analytes

HGH and TGAB showed 95CI% significantly different from B% only when serum was compared to plasma. It must be noted that very large %bias were observed for TGAB on these two comparisons. In contrast, Ferritin showed significant differences only

Table 4. Serological analytes. For each comparison was shown: the number of tests (n), the BA Bias calculated as percentage (%bias), the confidence interval (CI95%) and the biological variation expressed as desirable specification for inaccuracy (B%) (18). Analytes with a 95CI% exceeding B% are highlighted in grey.

Analyte	n	SST vs Barricor		SST vs PST		Barricor vs PST		B%
		%bias	CI95%	%bias	CI95%	%bias	CI95%	
ANTIS	64	10.4	6.23, 14.64	9.9	6.60, 13.46	-1.2	-2.82, 0.33	5*
CMVG	64	2.0	-0.90, 3.01	2.3	-1.53, 3.04	0.4	-1.95, 1.14	5*
CMVM	60	1.9	1.05, 2.79	2.2	1.07, 3.30	0.2	-0.52, 1.01	5*
RUBEOG	63	-0.1	-0.72, 0.61	-0.4	-1.11, 0.35	-0.3	-0.36, 0.85	5*
RUBEOM	61	0.0	-0.72, 0.72	0.1	-0.69, 0.93	0.1	-0.59, 0.54	5*
TOXOG	66	0.1	-0.39, 1.78	0.1	-2.39, 1.87	0.0	-0.88, 2.73	5*
TOXOM	58	-0.3	-0.97, 0.38	-0.8	-1.39, 0.01	-0.6	-0.06, 1.20	5*

when BAR was used whereas, for vitD3, only when PST was used. CTX showed a 95CI% significantly different from B% only when SST was compared to BAR. E2, TPO and PROG showed 95CI% exceeding B%, and containing the zero, in all of the three comparisons.

3.3. Serological analytes

All of the analytes showed 95CI% smaller than the arbitrary adopted 5% threshold, with the exception of ANTIS which showed a 95CI% significantly different from B% when serum was compared to either PST or BAR.

4. Discussion

Among the biochemical analytes BIC, K, LAD, LPA, P and TP are clearly associated to a matrix effect which was considered clinically significant (table 5). The TP and K differences between plasma and serum were expected and attributed to the coagulation process (19). Calcium and GLU showed both a matrix effect and an influence of the new BAR mechanical separator. In the case of GLU the two effects add up when SST is compared to BAR whereas for Ca they have opposite signs. Furthermore the B% for Ca was so small (0.8%) that, although the %bias were significant, we considered them clinically irrelevant (table 5). The same was true for Na and TRF (B%: 0.2 and 1.3% respectively) which showed matrix effects and influences of the new mechanical separator so small as to be considered clinically irrelevant (Table 5).

PCT showed %biases higher than 30% in the SST vs PST and BAR vs PST comparisons (likely arising from the low concentration data associated with the healthy condition of the individuals tested) whereas a %bias lower than the arbitrary adopted 5% was observed in the SST vs BAR comparison. However, in the latter comparison the 95CI% contained the zero and exceeded the desirable specification interval. Thus, we cautiously did not state whether the two measurements were equivalent or not (Table 5).

Among the immunochemical analytes HGH and TGAB were associated to a matrix effect only. Because of the very large %biases and 95CI% observed for TGAB, we prudently did not draw any conclusion for these measurements. We might speculate that the large %biases were consistent with the Roche recommendation for the exclusive use of serum for TGAB determination (table 5). For CTX the matrix effect (SST vs PST) and the mechanical separator effect were both insignificant. However the two effects adds up resulting in a significant difference between SST and BAR (Table 5). A significant difference was observed for Ferritin as well which showed no matrix effect but a pronounced effect of the mechanical separator (Table 5). In contrast, the matrix effect and the effect of the mechanical separator (both significant) observed for vitD3 were of the opposite sign. As a result SST and BAR can be used interchangeably, for vitD3 measurements, whereas re-

Table 5. Differences observed when comparing SST, PST and BAR tubes. NS: no statistically significant difference; SS-CLI: statistically significant difference and likely clinically relevant (highlighted in grey); SS: statistically significant difference only, clinically irrelevant

Analyte	SST vs BAR	SST vs BAR	BAR vs PST
Biochemical: ALB, ALP, ALT, AMS, AMSP, ASO, AST, B2MICR, BILT, C3, C4, CHE, CK, CL, CHO, CREA, CRP, FE, GGT, HOM, HDL, IGA, IGG, IGM, LIP, LDL, MG, MUCO, RF, TG, UA, UREA	NS	NS	NS
BIC	SS-CLI	SS-CLI	NS
Ca	SS[a]	SS[a]	SS[a]
GLU	SS-CLI	SS-CLI	SS-CLI
K	SS-CLI	SS-CLI	NS
LAD	SS-CLI	SS-CLI	NS
LPA	SS-CLI	SS-CLI	NS
NA	NS	SS[a]	SS[a]
PCT	?[b]	?[b]	?[b]
P	SS-CLI	SS-CLI	NS
TP	SS-CLI	SS-CLI	NS
TRF	NS	SS[a]	SS[a]
Immunochemical: AFP, B12, BHCG, CA125, CA153, CEA, CKMB, CORT, CPEP, Folate, FSH, FT3, FT4, GICA, GLA, HPRL, HS-CTnT, INS, LH, MIOG, PROPNB, PSA, T3, T4, TESTO, TSH	NS	NS	NS
CTX	SS-CLI	NS	NS
E2	?[b]	?[b]	?[b]
Ferritin	SS-CLI	NS	SS-CLI
HGH	SS-CLI	SS-CLI	NSD
PROG	?[b]	?[b]	?[b]
TGAB	?[b]	?[b]	NS
TPO	?[b]	?[b]	?[b]
vitD3	NS	SS-CLI	SS-CLI
Serology: CMVG, CMVM, RUBEOG, RUBEOM, TOXOG, TOXOM	NS	NS	NS
ANTIS	SS-CLI	SS-CLI	NS

[a]Because of the relatively small desirable specification (B%), the %bias, although significantly different, was considered clinically irrelevant.
[b]We, cautiously, did not state whether the two measurements were equivalent or not.

placing SST with PST might have significant clinical implications (Table 5). For E2, PROG and TPO the plasma vs serum comparisons all gave confidence intervals which exceeded B% and, at the same time, contained the zero. This was likely the consequence of the many results falling in the low concentration range and associated with the healthy condition of the individuals tested (Figure S2A-B). Thus we, cautiously, did not state whether the measurements were equivalent or not.

B% was not available for the serology analytes thus an arbitrary 5% threshold was adopted. Among them only ANTIS showed a significant matrix effect which was considered clinically significant (table 5).

5. Conclusion

We demonstrated that plasma tubes, including the new BAR tube, can be used interchangeably with SST for most of the standard biochemical and immunochemical analytes as well as for serology analytes. This is of particular importance because using the same tube for assaying multiple analytes significantly increases the efficiency and effectiveness of the laboratory while decreasing patient discomfort.

For the few analytes showing clinically significant between-tubes differences (Table 5), a new normal clinical range should be calculated in order to guaran-

tee the patients' safety. It must be also noted that the results showed in this study refers to a Roche COBAS 8000 device and its related assays. Thus, laboratory equipped with different instrumentations might show different outcomes.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Serum irisin levels as a potential marker for diagnosis of gestational diabetes mellitus

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Summary. *Objectives:* The aim of this study was to compare serum irisin, trace elements (Zn, Cu, Mg) levels between the group of pregnant women with gestational diabetes mellitus (GDM) and healthy pregnant group. *Material and methods:* Sixty pregnant women with GDM and 30 healthy pregnant women. The two groups were matched for maternal age, gestational age. Maternal serum irisin levels were measured by enzyme-linked immunosorbent assay kit at 24-28 weeks of gestation. An association between maternal serum irisin levels and clinical and biochemical parameters was evaluated. Body mass index, serum levels of glucose, OGTT, insulin, HbA1C, HOMA IR, HOMAβ, Hb%, and irisin were investigated and analyzed in the study group and controls. *Results:* Pregnant women with GDM had significantly higher fasting blood glucose FBG (p = 0.004), first-houEr OGTT glucose (p = 0.001), second-hour OGTT glucose (p = 0.001), fasting insulin FI (p = 0.001) levels, HOMA IR (p = 0.001), HOMAβ (p = 0.001), HbA1C(p = 0.001), Hb% (p = 0.017), as compared to controls. serum irisin levels were significantly lower (p =0.001) in women subsequently developed GDM (mean ± SD =71.65±8.03) than healthy pregnant controls (mean ± SD 136.54±22.56). Correlation analysis between irisin levels and anthropometric and biochemical parameters in patients with gestational diabetes revealed that none of the investigated parameters correlated with serum irisin level. *Conclusions:* The present results suggest that serum irisin levels might presented as a novel marker for GDM, with decreased levels of irisin being symptomatic of GDM. (www.actabiomedica.it)

Key words: gestational diabetes mellitus, irisin, trace elements, glycemic indices

Introduction

Gestational diabetes mellitus (GDM) is defined as a carbohydrate intolerance of varying severity, with onset or first recognition during pregnancy. GDM prevalence may range from 1% to 14% of all pregnancies, depending on the studied population and the diagnostic test employed (1). The pathogenesis of GDM is multifactorial and may include genetic and environmental factors, but the exact mechanism remains to be fully elucidated (2). Women with GDM are at increased risk of perinatal morbidity, impaired glucose tolerance, and type 2 diabetes in the years after pregnancy. Women with this condition may or may not have diabetes before. This condition goes away after delivery. When a woman is diagnosed with gestational diabetes mellitus, there is a risk of having it in the future pregnancies. Women who had this condition during pregnancy are more likely to develop Type 2 diabetes mellitus (3). Human gestation is characterized by weight gain and a progressive decrease in insulin sensitivity, which parallels the growth of the fetoplacental unit. Maternal insulin resistance in late gestation is an important mechanism to divert nutrients to the fetus to promote growth (4). Normal gestational insulin resistance is further enhanced in pregnancy complications, such as those resulting in abnormal fetal growth i.e. fetal macrosomia and intrauterine growth restriction (IUGR). Recent compelling evidence suggests that these pregnancy disorders are associated with

future development of maternal metabolic syndrome (5, 6). Insulin resistance plays an important role in the pathogenesis of GDM and despite extensive research, the mechanisms underlying insulin resistance are not fully understood (7). Insulin resistance in pregnancy is traditionally attributed to increased maternal adiposity and placental hormones with diabetogenic action (8, 9), although the underlying mechanisms are not fully understood. Recent investigations have focused on several new potential mediators of gestational insulin resistance (9). Since it is the largest organ in the body, skeletal muscle accounts for the majority of glucose uptake in response to insulin, and is quantitatively the most important site of insulin resistance. During the past decade, skeletal muscle has also been identified as a secretory organ and cytokines and other peptides produced and secreted by myocytes are classified as myokines (10). These myokines function as endocrine hormones and regulate the function of various distant organs.

Irisin is a novel myokine (1), adipokine (2) and neurokine (3) consisting of 112 amino acid residues, with a molecular weight of 12 587 kDa (11,12). Proteolytically processed from the product of fibronectin type III domain containing 5 (FNDC5) gene in response to the activation of peroxisome proliferatoractivated receptor g (PPARg) co-activator-1a (PGC-1a) (11) and is an anti-diabetic hormone that regulates the glucose metabolism and energy consumption via converting white to brown adipose tissue (13).

Recently has been identified as an exercise-induced hormone secreted by skeletal muscle and has been proposed to mediate the beneficial effects of exercise on metabolism (14). Sedentary lifestyle is a major risk factor for type 2 diabetes mellitus. Randomized controlled trials have demonstrated that physical activity improves glucose tolerance and reduces the risk of type 2 diabetes mellitus (15). Therefore, it has been speculated that physical exercise may exert its beneficial effects on energy metabolism through secreted factors from myocytes such as irisin (16). Recent studies have shown that circulating irisin levels were significantly lower in patients with type 2 diabetes compared to people without diabetes (17, 18).

Studies in mice have shown that FNDC5 which directly stimulates the conversion of white adipose tissue (WAT) to brown-like adipose tissue (BAT), leading to increased total energy expenditure and, subsequently, to weight loss, improved glucose tolerance and insulin sensitization (19). Due to its metabolic properties, irisin has recently attracted a lot of interest as a potential new target for the treatment of obesity and its associated disorders. In clinical settings, circulating irisin levels are reportedly lower in patients with obesity and type 2 diabetes mellitus (DM) (20, 21), indicating that irisin may play an essential role in glucose intolerance. However, circulating irisin is reported to be paradoxically higher in adults with the metabolic syndrome (22), suggesting that states of irisin resistance or tolerance may exist (23). Data regarding irisin in human pregnancy are scarce. Irisin precursor is expressed in human placenta during gestation and its serum levels are higher during the entire pregnancy, when compared with nonpregnant women. After adjusting for body mass index (BMI), maternal irisin levels were associated with the homeostasis model assessment of estimated insulin resistance, suggesting that irisin may contribute to thedevelopment of normal gestational insulin resistance (24).

Objective

The aim of the study was to compare Serum irisin concentrations between pregnant women with GDM and healthy pregnant women. irisin levels may have a potential as a novel marker for diagnosis and follow-up of gestational diabetes mellitus.and also to evaluate the correlations between Zn2+, Cu2+and Mg2 and alteration in serum irisin concentrations between pregnant women with GDM and healthy pregnant women.

Materials and methods

The case-control study was conducted at Pegnant care center, AL-Najaf province, Iraq, between June 2017 and March 2018. The Ethics Committee of the institution approved the study, and all participants provided informed consent. The study group comprised 60 women diagnosed with GDM and 30 healthy pregnant controls with normal oral glucose tolerance test

(OGTT) results. All participants were recruited at the time of screening for GDM using a 75 g, 2-h OGTT between 24 and 28 weeks of pregnancy. GDM was diagnosed when one or more abnormal plasma glucose values (fasting_92 mg/dL, 1h_180 mg/dL, 2 h_153 mg/dL) were obtained using the criteria of The International Association of Diabetes and Pregnancy Study Groups (25).

The GDM and control groups were matched for maternal age, gestational age and current body mass index (BMI). Gestational age was determined by the last menstrual period and confirmed by ultrasonographic examination performed during the first trimester of pregnancy. BMIs measured during OGTT screening using the following formula: weight (kg)/height (m2). No patients received medications that interfered with glucose or lipid metabolism before blood sampling. Patients with multiple pregnancy, pre-existing glucose intolerance, pregnancy-induced hypertension, preeclampsia, acute or chronic inflammation, as well as active smokers were not included. An overnight fasting venous blood sample was obtained from all participants to assess Iris in levels and other biochemical parameters on the day of OGTT screening. All samples were stored at room temperature for at least 30 min to allow the blood to clot, followed by centrifugation (3000 rpm) for 15 min to separate serum. Serum specimens were aliquoted and stored at _80 _C until Iris in levels were analyzed. Glucose levels during OGTT were measured with the hexokinase method using a commercially available kit (Beckman AU5800; Beckman Coulter Diagnostics, Brea, CA). Insulin levels were determined using a chemiluminescent assay (AccessDxI800; Beckman Coulter Inc., Brea, CA), and glycosylated hemoglobin (HbA1c) levels were determined using commercially available kits and high-performance liquid chromatography (Tosoh HLC 723 G8, Tosoh Bioscience, Tokyo, Japan). Serum triglyceride, total cholesterol, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterollevels were determined using an autoanalyzer (Beckman AU5800; Beckman Coulter Diagnostics, Brea, CA). Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using the following formula: fasting glucose (mmol/L)_fasting insulin (IU/mL)/22.5 (26). Serum concentrations of magnesium, zinc, and copper were measured by colorimetric method using Randox kit (Randox, UK).

Statistical Analysis

Statistical analysis was performed using two statistical software, the Statistical Package of Social Science (SPSS ver. 21) and Graphpad Prism ver.5. Continuous variables were expressed as mean ± standard deviation (SD). Significant differences were assessed using Paired t-test and independent t-test for variables with equal and unequal frequencies respectively. Bivariate correlations were assessed using standardized Pearson coefficients. The *p* values obtained of less than 0.05 and 0.01 were considered as statistically and highly statistically significant respectively.

Results and discussion

The demographic characteristics of all participants are shown in Table 1, The total study population was 60 gestational diabetes mellitus, 30 normal pregnant in each group. The mean of maternal ages, and gestational ages of the two groups were not significantly (NS) different. In addition, BMI at the time of sample collection were differ in both groups.

Comparisons of clinical data between the two groups are presented in Table 2. In the fasting glucose, OGTT, insulin, and HOMA-IR values (p = 0.001), also Hb% (p = 0.017) were significantly higher, except HOMAβ (p = 0.001) were significantly lower in the GDM group in comparison to control at the time of GDM screening serum irisin levels were significantly

Table 1. The demographics characterizes of the study population

Variables	GDM	Control	P value
Mother's age (Years)	26.33±3.21	26.07±3.58	0.772 NS
BMI Kg/m² at sampling	34.39±2.00	31.04±1.82	0.000**
Gestational age (weeks) at sampling	28.47±0.96	28.07±0.98	0.069 NS

BMI: body mass index

Table 2. Clinical characteristics of the healthy pregnant controls and women diagnosed with GDM

Parameters	GDM Mean ± SD	Control Mean±SD	P value
Glucose(mg/dl)	115.35±11.82	99.00±15.37	0.000**
OGTT(mg/dl) 1h	182.04±4.23	133.23±5.22	0.000**
OGTT(mg/dl) 2h	149.38±8.19	103.11±2.15	0.000**
Isulin(µlu/ml)	15.32±2.70	8.63±1.20	0.000**
HOMA IR	2.51±0.22	1.97±0.21	0.000**
HOMO β	103.00±23.86	84.70±6.92	0.000**
HbA1C%	5.08±0.23	4.47±0.19	0.000**
Hb %	11.70±0.77	11.27±0.81	0.017*
Irisin(ng/ml)	71.65±8.03	136.54±22.56	0.000**

OGTT: oral glucose tolerance test, HbA1c: Glycated heamoglobin A1c, Hb: Hemoglobin

lower ($p < 0.001$) in women subsequently developed GDM (mean ± SD =71.65±8.03) than in controls (mean ± SD 136.54±22.56).

Relationships between serum irisin levels and other variables analyzed independently at the time of GDM screening are presented in Table 3. In the GDM group, no significant correlations were observed between serum irisin levels and other clinical or biochemical parameters.

As shown in Table 4, serum Zn levels were significantly lower in GDM women as compared to normal pregnancy (p=0.001) However, a significantly serum Cu lower level was observed in the healthy pregnant compared to GDM group (p=0.001).Conversely, serum Mg levels significantly lower was observed in GDM group were compared to healthy pregnant women (p=0.001).

Table 3. Correlations between irisin levels with other biochemical parameters in control subjects and in women diagnosed with GDM

Parameters	R	P-value
Glucose(mg/dl)	-0.240 NS	0.065
OGTT(mg/dl)1hr.	-0.232 NS	0.074
Insulin(µlu/ml)	-0.038NS	0.774
OMMA IR	-0.154 NS	0.241
HOMMA β	0.044 NS	0.740
HbA1C(%)	-0.077 NS	0.558
Hb(%)	0.038 NS	0.773

Table 4. Comparisons of trace Elements in patients with gestational diabetes mellitus and control group

Parameters	Groups	Mean±SD	P value
Cu (µg/dl)	GDM	109.00±14.62	0.001**
	Control	85.43±5.06	
Zn (µg/dl)	GDM	79.27±6.87	0.001**
	Control	101.30±7.20	
Mg (mg/dl)	GDM	1.99±0.07	0.001**
	Control	2.35±0.07	

Gestational diabetes mellitus (GDM) is a metabolic disorder during pregnancy leading to acute and chronic complications in both mother and newborn. Thus, GDM patients have an increased risk of co-morbidities during pregnancy, e.g. preeclampsia, pregnancy-induced hypertension, and shoulder dystocia with impeded delivery (27). Furthermore, chronic complications might occur after delivery including type 2 diabetes mellitus (T2DM) and cardiovascular disease (28, 29).

Therefore, early diagnosis and appropriate treatment of GDM is helpful in reducing the adverse maternal and fetal outcomes and in protecting mothers and infants from long-term complications. Thus, previous studies have tried to determine the predictive value of maternal or placental biomarkers before the development of GDM, and these identified in many biological process involving insulin resistance, carbo-

hydrate metabolism, oxidative stress, and inflammation (30). To the best of present knowledge, this result are the first to use a case- control study to measure irisin in the serum of GDM patients and healthy controls in Iraqi population. Furthermore in the present conducted this analysis to evaluate the circulating irisin between GDM patients and healthy pregnant. Consistent with these findings, this study confirmed that pregnant with GDM have lower circulating irisin. implicated in the maternal metabolic disturbances associated with abnormal fetal growth. Pregnancy is associated with substantial changes in maternal metabolism, which provide sufficient energy and nutrients to the fetus (31, 32). In this context, mothers develop a state of insulin resistance during midpregnancy, which progresses throughout the third trimester, leading to reduced consumption of glucose by maternal tissues and increased gluconeogenesis (31). However, in a substantial proportion of pregnancies, the insulin-resistant condition is greatly increased, resulting to adverse maternal metabolic state and fetal growth abnormalities (33-34).

Irisin is a novel myokine and adipokine which induces an increase in total body energy expenditure, improving insulin sensitivity and glucose tolerance in experimental animals. In the present study showed that serum irisin levels were significantly lower in the patients with GDM than in the healthy pregnant women, the present results are agreement with the findings of Yuksel et al. (35), who also reported a decrease in circulating irisin in women with GDM; however, Kuzmicki et al. found that serum irisin increased significantly in pregnant women, but this increase was significantly lower in subjects with GDM (36). The concentration of irisin increased significantly from colostrum to transitional and mature milk, and plasma irisin also increased in lactating women with and without GDM compared to non-lactating women (37). In contrast, Ebert et al. (38) In GDM, there is enhanced ability of glucose to cross the placenta, with resultant fetal hyperglycaemia, hyperinsulinaemia and macrosomia. This may lead to a variety of fetal pathologies postpartum and pregnancyassociated morbidity, such as preeclampsia (39-40) and susceptibility to development of GD in subsequent pregnancies. Up to 90% of GDMafflicted women develop type 2 diabetes (41). GDM may therefore, serve to unmask women who are predisposed and destined to develop type 2 diabetes later in life (42) - found no difference in circulating irisin between pregnant women with and without GDM, although 4 years after childbirth irisin levels were significantly higher in patients with previous gestational diabetes mellitus than in women with normal glucose tolerance. Conversely, Aydin et al. and other studies (37, 43, 44) showed lower serum irisin in lactating women with GDM in comparison with healthy lactating women. No significant differences in serum irisin between non-obese, obese and GDM subjects at term were recently reported by Piya et al. (45). However, further studies revealed that after adjusting for BMI, lipids and glucose, irisin levels were significantly lower in non-obese pregnant women as compared with obese and GDM groups. Our results showed that irisin levels were markedly lower than healthy pregnant, disagreement with other studies which may suggest a compensation for a physiologic increase in insulin resistance or a stimulating effect of high estrogens levels (46). or possibly its additional secretion by the placenta, although the influence of placental tissue to circulating irisin appears insignificant (46, 47). The authors suggested that these findings may reflect irisin resistance developing together with insulin resistance.

The concept of irisin resistance with compensatory hyperirisinemia was also proposed by Hee Park et al. (48), who showed that high irisin levels were associated with an increased risk of the metabolic syndrome and cardiovascular disease. However, an association between irisin and insulin resistance, in particular during pregnancy, seems still unclear. Piya et al. (49) confirmed that in pregnant women serum irisin was positively correlated with fasting blood glucose, insulin and HOMA-IR. Ebert et al. (47) In contrast, Yuksel et al. (43). reported that serum irisin level was negatively correlated with HOMA-IR in individuals with various degree of obesity. Additionally, we observed that in the whole group of pregnant women serum irisin concentration correlated negatively with glucose level at 120 min of the OGTT, which is consistent with the results of Choi et al. (50), who found that 2 h plasma glucose was an independent negative predictor of irisin concentration in the patients with newly-diagnosed type 2 diabetes.. All these discrepancies may

result from differences in clinical characteristics of the subjects studied and various diagnostic criteria; however, the potential effect of BMI or weight gain during pregnancy and gestational week at sampling appears controversial since a positive correlation between irisin level and body mass index at the last weeks in third trimester of gestation (47) and a negative one at term (49), were reported by different authors. In the present study, no associations between circulating irisin and BMI were observed. Moreover, controversial results, i.e. higher irisin concentration in pregnant than in non-pregnant women (47) or no significant differences during and after pregnancy (49), have been found in different studies.

Results of trace elements

In the present study suggest that this element also contributes at some level to the pathogenesis of GD and pregnancy in diabetes. This is consistent with the role of this metal as a regulator of carbohydrate metabolism in pregnancy (51) The effect of diabetes in pregnancy may arise through two related mechanisms, namely, the direct effect of trace elements and oxidative stress on immune regulation (52). A significant decrease in Zn concentration was shown in the diet-treated diabetic group relative to healthy pregnancy which supports the hypothesis that Zn and Cu may play a role in the mechanisms regulating the immune response (53, 54).

Another study found that deficiency of Mg++ is associated with immunosuppression in athletes, suggesting that Mg++ has a role in immunoregulation (55, 56).

Magri et al. did not find a relationship between the serum levels of calcium, magnesium, and zinc and gestational hypertension, therefore, they proposed that these elements might not clinically participate in the pathogenesis of the gestational hypertension (57). The mean serum levels of magnesium, copper and zinc between the two groups were significantly different. For defining, the role of serum electrolytes in GDM more research is necessary. The results of the present study showed that these elements did play a prominent role in the pathogenesis of GDM.

Conclusions

Maternal serum irisin levels of patients with GDM are significantly lower compared with healthy pregnant as controls. However, The present results suggest that serum irisin levels might presented as a novel marker for GDM, with decreased levels of irisin being symptomatic of GDM, and revealed that these trace elements Cu,Zn,Mg did play a conspicuous role in the pathogenesis of GDM.

The important issues of the associations between maternal insulin resistance during pregnancy, and future risk of the metabolic syndrome in mother need to be further addressed in future prospective studies.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Intraoperative neuromonitoring in traditional and miniinvasive thyroidectomy. A single center experience in 1652 nerve at risk

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Summary. *Background:* The world is rapidly urbanizing, causing alarming health problems to their citizens. The Cities Changing Diabetes program aims to address the social factors and cultural determinants that can increase type 2 diabetes (T2D) vulnerability among people living in cities. *Methods:* Public data of Italian Institute for Statistics (ISTAT) and available scientific reports were reviewed and findings integrated. The prevalence of T2D in the 8 health districts of Rome was mapped and the correlation between prevalence and social and cultural determinants was assessed. *Results:* The metropolitan area of Rome has 4.3 million inhabitants. People over 65 has increased by 136,000 units in the last decade, reaching 631,000 citizens in 2015. Elderly people living alone are 28.4%. The obesity prevalence is 9.3%, as compared to 8.2% in the year 2000. The prevalence of T2D is 6.6%, varying in the different 8 health districts between 5.9% and 7.3%. A linear correlation exists between the prevalence of diabetes in the districts, unemployment rate and use of private transportation rate (Pearson R 0.52 and 0.60, respectively), while an inverse correlation is present with aging index, school education level, and slow mobility rate (Person R -0.57, -0.52, and -0.52, respectively). *Conclusions:* Important socio-demographic changes have occurred in Rome during the last decades with a raise in the prevalence of obesity and diabetes. A wide variation exists in the prevalence of T2D among the districts of Rome, associated with social and cultural determinants. This study model can help rethinking diabetes in an urban setting. (www.actabiomedica.it)

Key words: thyroidectomy, videoassisted thyroid surgery, MIVAT, I-IONM, C-IONM, vocal cord palsy, dysphonia

Introduction

The injury of laryngeal recurrent nerve (RLN) is one of the most severe adverse event in thyroid surgery. The rate in literature is reported as 2-11% for transient palsy and 0,6-1,6% for permanent palsy (after 6 months from surgical procedure) (1).

In the last years the use of Intraoperative Nerve Monitoring (IONM) is improved using different devices in order to identify the correct elettromiographic (EMG) signal; in our practice we used the NIM-Response 3.0 System (1-2).

The IONM technique combines the anatomical evaluation of RLN, gold standard in thyroid surgery with the EMG signal. There are several advantages in using the combined technique, such as facilitation in identification, localization and distribution of the RLN and reduction of the incidence of RLN damages (Table 1). These specific procedures require a collaboration between surgical and anesthsiological team.

Table 1. The advantages of the combined technique (anatomical evaluation and elettromiographic signal)

Specific advantages
1. Facilitates the identification, localization and distribution of the RLN
2. It allows the identification of possible anatomical variants of the RLN
3. Facilitates the exposure and dissection of the RLN
4. It is useful for the completion of the thyroid resection
5. Facilitates the evaluation of functional integrity and eventually the recognition of nerve damage mechanisms
6. Reduces the incidence of damages of the RLN
7. Facilitates learning for unskilled. surgeons in training, as it guides them to nerve identification
8. It is also useful for experienced surgeons in bilateral thyroid surgery

The anesthesiologist plays a role during the NIM use, especially in the selection of drug for the induction and maintenance of anesthesia and in the correct placement of endotracheal tube (ET) (3).

Currently, RLN lesions can be classified into segmental lesion (Type 1) and global lesion (Type 2) (2).

Recent studies report that more than 70% of lesions appear to be of type 1, defined as the interruption of RLN conduction at a specific point or segment. Usually this type of injury is related to traction, clamping, compression and/or thermal spread damage (4-5).

A normal function of the RLN evidenced by the presence of laryngeal contraction, in the absence of the EMG signal is defined as false LOSS. This is the situation in which there is no damage at the level of the exposed region of the RLN and no response from the contralateral nerve stimulation is elicited. False signal losses may result from incorrect functioning of the equipment, or erroneous placement of the ET tube equipped with the electrodes. Uncorrect placing or misplacement of ET tube can also be caused by excessive traction on the trachea, especially during thyroidectomy for great goiter (6).

The aim of this study was to describe the frequency of the injury of RLN in our hospital during thyroid surgery, in which the IONM was applied.

Materials and methods

In this retrospective study, approved by Ethics Committee of Parma, the patients undergoing thyroid surgery associated to IONM have been enrolled consecutively from 21/08/2014 to 30/08/2018. Patient data was collected in a database.

Patient data is related to :

• Age

• Sex

• Type of surgery (thyroidectomy, lobectomy)

• Type of thyroid pathology (Thyr3, Thyr 4, Thry 5 or Thyr 6 nodules considering the Bethesda System for Reporting Thyroid Cytology, Basedow-Graves disease, Medullary Carcinoma and uninodular or multinodular goiter)

• Pre-operative clinical symptomatology, ENT evaluation highlighting dysphonia, dysphagia, dyspnoea.

• Post-operative symptomatology.

Data collected also concern the type of device used, i.e. NIM Response 3.0 (Medtronic Xomed, Jacksonville, Florida, USA) with intermittent monitoring system (I-IONM) and stimulation specifications; in particular:

1. Amplitude of the Vagus nerve (VN) and RLN as standardized protocol

2. Signal anomalies.

We collected data from I-IONM and not by Continuos IONM because we have interrupted C-IONM after a case of cardiac arrest (15TH patient) during the APS placement.

The statistical software used for data analysis was SPSS.

The utility of the intraoperative neuro-monitoring system was evaluated with the following statistical methods:

• Mann Withney U-test with independent samples is used to evaluate any existing relationships between pre-operative symptomatology and VN and RLN pre dissection voltages (ie V1, RLN 1 according to the guidelines of the IONM Group) and for evaluate the relationships between post-operative symptomatology and VN and RLN post dissection voltages, comparing with asymptomatic cases.

• T-samples with coupled samples has been applied

to assess if there was a specific trend of VN pre-dissection and VN post-dissection voltage in symptomatic patients, making a comparison with asymptomatic cases.

• Wilcoxon signed sign test has been used to related correlated samples to assess if goiter pathology could result in a statistically significant NV and RLN values variation pre and post dissection.

Chi square test to asses the rate of vocal cord palsy in traditional and videoassisted procedure.

• Positive predictive value (PPV) expresses the probability that a patient with intraoperative evidence of disappearance of the electromyographic signal at neuromonitoring is really affected by a nerve injury;

• Negative predictive value (NPV) expresses the probability that a subject with intraoperative evidence of electromyographic signal is really not affected by a nerve injury.

We considered the values for $p < 0.05$ statistically positive.

Results

We collected data on 928 consecutive patients, 689 were female and 239 male, ratio of 3: 1.

The mean age was 55.14±13.5 years (range 15-89).

The mean age in relation to gender was 53.51±13.6 years in the female and 56.03±13.5 years in the male.

On 928 cases, 54 patients were treated for Basedow-Graves disease, 388 for goiter, 59 cases for thyr 3, 167 cases for thyr 4, 43 cases thyr 5, and finally 215 treated for Thyr 6 nodule. We identified also 2 cases treated for medullary cancer and lymphoadenectomy (Table 2).

The surgical procedure were 204 lobectomy , 724 total thyroidectomy. 129 cases were treated with minimally invasive video assisted technique (61 lobectomy; 68 total Thyroidectomy) (7, 8). We identified 1652 nerves at risk (Figure 1).

We have analyzed separately the cases with preoperatively symptomatology compatible with hoarseness in the absence of positive ENT evaluation (no cordal hypomotility).

Table 2. Surgically treated cases

Thyroid disease	Treated cases
Thyr 3	59
Thyr 4	167
Thyr 5	43
Thyr 6	215
Goiter	388
Basedow	54
Medullary	2

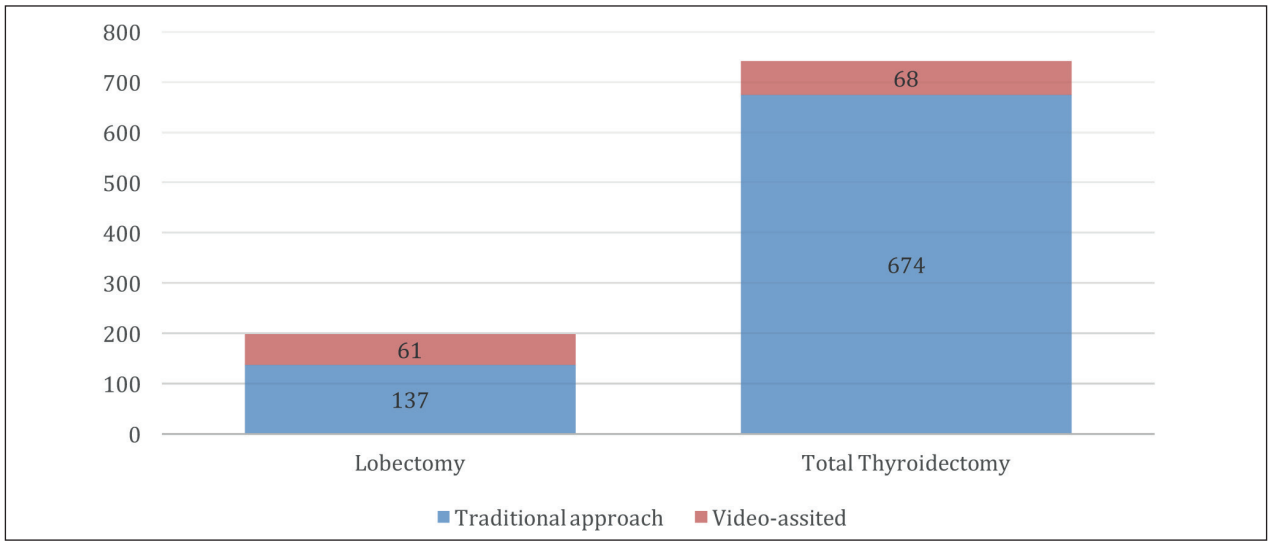

Figure 1. Surgical procedures performed

No relationship between preoperative symptom and voltage at initial stimulation (pre-dissection RLN and VN voltage) has been identified; even the distribution of voltages (right VN pre-dissection, left VN pre-dissection, right RLN pre-dissection and left RLN pre-dissection) was found not to be different between these patients and the group without preoperative symptomatology.

The same procedure was applied to cases with a pre-operative symptomatology compatible with dysphagia, dyspnea and compression, obtaining the same results.All patients were treated with traditional thyroidectomy.

The symptoms reported after surgical procedure were then assessed (focusing mainly on dysphonia, dysphagia, dyspnoea). We analyzed cases that showed a symptomatology compatible with dysphonia without intraoperative signal loss; it was observed that no relation related to postoperative symptom and voltage can be proved in these cases. From this analysis, obtained with Mann-Whitney U test application, the same distribution was obtained in symptomatic and asymptomatic patients.

The same results were obtained in case of occurrence of postoperative dyspnoea and dysphagia.

Using the neuromonitoring procedure 8 cases of signal loss occurred during the dissection of the first lobe in course of total thyroidectomy; in 2 cases it was found to be related transitory dysphonia, in 5 cases to technical problems, in 1 case in patient with pre-existing cordal paresis.

It was necessary perform a two-stage thyroidectomy in 7 cases.

In patients who were candidates for total thyroidectomy, we recorded the loss of the signal after the second lobe in 20 cases, of which 9 cases showed dysphonia in the post-operative period(Ent evaluation after 48 hours).

The sensitivity and the specificity of the instrument are respectively equal to 100% and 98%.

The PPV (positive predictive value) was found to be about 52%. Instead the NPV (negative predictive value) turned out to be 100%. We recorded a transitory dysfunction in 16 cases equal to 0,9% and definitive in 7 cases equal to 0.4% after 6 months with an ENT revaluation.

No case bilateral dysphonia is recorded.

Analyzing the traditional and videoassited thyroidectyomy we report 2 cases of transitory dysfunction in 129 MIVAT and 14 cases in Traditional Thyroidectomy (p=ns); 1 persistent palsy in MIVAT and 6 cases in traditional thyroidectomy (p=ns).

Discussion

In recent years there has been a progressive increase in the use of IONM both during procedures with open standard technique and during MIVAT (5).

The increase of application of IONM is related to a *feel safety* from surgeon during surgical procedure. IONM helps the surgeon in localization and identification of the RLN. IONM permits to evaluate its functional integrity (in addition to the classical anatomical integrity).

Neuromonitoring would constitute an improvement to thyroidectomy. Moreover, this device seems to have an application to reduce the rate of transitory and permanent paralysis of the RLN.

Through our study it is not possible to identify a relationship between pre-dissection voltages and non-certified ENT pre-operative symptomatology, it is not possible to find a relationship between post-dissection voltages and post-operative symptomatology (compression, disphagya) unrelated to cordal hypomotility. It is not possible to find a specific variation of the voltages from the pre-dissection to the post-dissection that allows to predict the postoperative symptomatology, except a PPV of 52% in case of nerve injury stupor.

IONM is extremely valuable tool to reduce the percentage of hoarseness. In this research, it was found to be <1%. It also helps the surgeon to choose the intraoperative strategy, reducing the incidence of bilateral paralysis to zero. In case of loss of the signal during the dissection of the first lobe, it is necessary to proceed firstly with the assessment of lesion site, following the different steps provided by the resolution algorithms. A two-stage thyroidectomy should be performed to eliminate this risk of bilateral paralysis that could result from the continuation of the procedure on the second lobe. In our study there were 8 loss of signal during the dissection of the first lobe (5 of which were

attributable to technical problems), which led to the need to adopt this scheme in 7 patients, performing a two-stage thyroidectomy.

In high-volume centers the rate of transitory/permanent vocal cord palsy is low but also in these Units the need of *feel safety* is related to IONM. The use of the IONM is an helpful tool for dissection and anatomical identification of the nerves. IONM has a high specificity and sensitivity and a high negative predictive value. PPV as 52% definable low, may be related to the transient stupor of nerve function. In all patients a bolus of cortisone e.v. is administered intraoperatively and a rate of these patients can already recover in the early hours after lobectomy with negative feedback to the ENT visit after 48 hours.

From a medico-legal point of view, the neuromonitoring data can not yet be considered as objective evidence. To avoid legal disputes it is suggested to establish a good doctor-patient relationship; correct information about the pathology, the better treatment and potential consequences are crucial (9). We have to compile a detailed report of any procedure performed. If neuromonitoring is used, it is suggested to apply it following the standardized steps and to report the complete documentation of EMG signals derived from the stimulation of the VN and the RLN.

The CIONM technique is reported as an excellent aid for the surgeon during routine thyroid surgery, providing an excellent tool to prevent harmful maneuvers especially in complex cases (such as recurring pathologies with numerous adhesions, infiltrating tumors adjacent to the structures, stalking goons with mediastinal sinking or with tracheal deviation). CIONM is able to provide real-time information in an uninterrupted manner,-allowing a continuous evaluation. It is to be interpreted as a repeated stimulation that is induced by the probe positioned on VN. Every time you exercise any type of movement during the surgical procedure the EMG signal is registered. The possible advantage of C-IONM derives from its potential to monitor, simultaneously with the surgical maneuvers, step by step, the functional integrity of the vagus, of the RLN and trasmit abnormal EMG signal. We have interrupted our experience with C-IONM after the reported case of cardiac arrest after 28 cases tretaed; few cases are reported (10).

We think that the concept of C-IONM is the better form of stimulation but that we must improve the device, the intraoperative handling of VN to reduce the dissection and torsion. In a recent metanalysis authors underline that the IONM could reduce the incidence of RLN injury (11).

Conclusion

Our study confirmed a high specificity, sensitivity and NPP of the IONM application in thyroid surgery; however, a low PPV in our sample has been identified, probably caused by transient stupor of nerve function.

In particular, no correlation between pre-dissection voltages of VN and RLN and preoperative symptomatology, characterized by a negative ENT evaluation, has been found.

We think that the use is indicated in all endocrine cervical surgical procedure, also in high volume centers, in research and teaching hospital.

Note: Compliance with ethical standards

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

A preliminary report on the use of Midodrine in treating refractory gastroesophageal disease: Randomized Double-Blind Controlled Trial

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Summary. *Background:* Gastroesophageal reflux disease (GERD) is a common disease with various clinical presentations. Acid suppression with proton pump inhibitors and lifestyle modification may not lead to satisfactory response in a substantial portion of patients. We investigated the possible effect of midodrine in patients with refractory GERD. *Methods:* Patients suffering from GERD and were refractory to one-month course of pantoprazole 40mg twice daily entered the study. This was a pilot, randomized, double-blind, and placebo-controlled study. After randomization, one group received Midodrine 5mg before meals for one month, and the other group received placebo for the same period. Meanwhile, pantoprazole was continued 40mg twice daily in both arms. The severity of symptoms was evaluated by the visual scoring system. Quality of life (QoL) in both groups was measured using a standardized version of Quality of Life in Reflux and Dyspepsia questionnaire (QOLRAD). *Results:* A total of twenty patients were enrolled in this study. There was a significant interaction between the groups and time on all measured scores based on QOLRAD questionnaire. All the markers in the Midodrine group had significant improvement over time, but the placebo group did not show any significant improvement. Both visual severity score and total QoL score in Midodrine arm showed a U shape change during 6 weeks. *Conclusions:* Midodrine before a meal could be useful in alleviating symptoms and improving QoL in the patients with refractory gastroesophageal disease. (www.actabiomedica.it)

Key words: gastroesophageal reflux, Midodrine-Hydrochloride, proton pump inhibitor

Introduction

Gastroesophageal reflux disease (GERD) is common with a variety of clinical presentations, imposing a considerable economic burden on patients and healthcare systems. Pathologic GERD occurs when reflux of stomach contents leads to heartburn, regurgitation, and/or complications due to chronic mucosal injury (1). Quality of life (QoL) of 40% of the population may be affected by this condition (2-5).

Despite good response to lifestyle modification and acid suppression with proton pump inhibitors (PPIs) in the majority of patients, there are still group of patients who are refractory to these measures. It is estimated that about 10-40% of patients do not show a satisfactory response to PPI. In addition to inad-

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equate dosing or improper timing of PPIs, there are other causes of refractory GERD including non-acid reflux, hypersensitivity, and eosinophilic esophagitis. The clinical definition of refractory GERD is controversial in the literature. Most experts consider refractory GERD in those who fail to show improvement in their symptoms, either partially or entirely, with PPIs twice daily (6-13).

The main pathogenesis of classical GERD, as well as refractory cases, are considered to be increased transient lower esophageal relaxations. A variety of medications might reduce this event including baclofen, lesogaberan, or acotiamide through various mechanisms (14-17).

These drugs have either significant side effects or in many cases, are not affordable. Midodrine is an alpha-1 adrenergic agonist with an excellent safety profile. Since alpha-adrenergic receptor stimulation causes lower esophageal sphincter contraction in physiologic studies, midodrine could theoretically reduce reflux episodes. For the first time, in this study, we investigated the possible effect of midodrine in managing patients with refractory GERD.

Patients and Methods

Patients

Patients who presumed to have refractory GERD referred to a special clinic affiliated to Shiraz University of Medical Sciences in 2015-2017. Before entering the study, all cases were given one-month course of pantoprazole 40 mg (ACTOVERCO Pharmaceutical Factory, Karaj-Iran under license of KRKA Company, Slovenia) twice daily. Those who completed this course of medication and their symptom failed to improve were considered for this study. Patients with the following criteria were deemed to be eligible: age 18-65 years, having had at least one symptom consistent with GERD (such as retrosternal burn or regurgitation), and lack of response to pantoprazole 40 mg twice daily for one-month. Exclusion criteria included prior abdominal surgery, dysphagia, significant comorbid illnesses that could interfere with the study or compromise patients' safety such as malignancy, peptic ulcer disease, hypertension(HTN), urinary retention, heart disease, vascular insufficiency, renal failure, pheochromocytoma, severe respiratory disorders, cirrhosis, hypersensitivity to midodrine, use of other medications affecting LES pressure or relaxation (including beta- blockers, steroids, theophylline, inhalers, antihistamines), any mental or psychiatric illness, pregnancy or breastfeeding in women, and consuming alcohol. Twenty patients were included in this study based on the inclusion criteria. All the patients signed a written informed consent before entering the study.

The histologic diagnosis of GERD is based on papers written in the early days of endoscopic biopsies, and the criteria described are still in use today. The typical features of GERD are increased thickness of the basal cell layer (thickness of the basal layer exceeds 15%); increased length of the papillae with suprapapillary thinning (extension above the midportion of the squamous mucosa); intraepithelial inflammation, including eosinophils, neutrophils and lymphocytes; and intercellular edema (spongiosis) (18, 19). The endoscopic diagnosis of GERD is based on erosive picture and experts seen erosion for all cases and just the patients with typical symptoms alone were included.

Study design

This was a pilot, randomized, double-blind, and placebo-controlled study (20, 21). The study was approved by the local Ethics Committee of Shiraz University of Medical Sciences, code number CT-P-92-6683. The study design and protocol was approved by the Iranian Clinical Trial Registry (IRCT) with identification # IRCT201402274226N2.

All twenty qualified patients underwent upper endoscopy at the beginning and biopsy was taken from the distal and mid part of esophagus following standard protocols. The participants were randomized using a computer-generated scheme into two groups with 10 cases each. All cases completed the study period. Both arms of the study were given pantoprazole 40mg twice daily. One group received 5mg midodrine tablets (Takeda pharmaceutical company, Linz-Austria, purchased from local market) three times per day before meals, and the other group was given a placebo with similar shape, packaging, and instruction for consumption for

four weeks. After completing the treatment course, all cases were followed for another two weeks while continuing to consume pantoprazole 40mg twice per day. All the participants were asked to complete a visual score of their symptoms severity from 1 to 10 (with higher scores indicating higher severity) at the onset of study (before drugs consumption), and at weeks 2, 4, and 6 (two weeks after drug cessation), successively.

Furthermore, all the patient filled out the verified and standardized Persian version of QoL in Reflux and Dyspepsia (QOLRAD) questionnaire at the beginning of the study (22), visits on weeks 2, 4, and 6, successively. This questionnaire assesses five different dimensions in GERD including emotional distress, sleep disturbance, food problems, physical/social functioning, and vitality. Each question scores from 1 to 7. score one shows low QoL, and a higher score indicates a better QoL. During all visits, blood pressure was measured. All women in childbearing age had a serum HCG test before being enrolled into the study and were asked to practice some forms of contraception during their participation.

Statistical analysis

All statistical analyses were done using statistical package for social sciences (SPSS) version 25. The repeated measure analysis of variance (RM-ANOVA) was employed to compare the changes during time between the groups. Within and between groups comparisons were done through the paired *t*-test and independent *t*-test. Chi-square test and Fisher exact test were used to compare qualitative variables between the groups. Qualitative and quantitative variables were described using frequency (percent) and mean ± standard deviation (SD). *P*-value less than 0.05 was considered to be statistically significant. Overall observed power (partial eta-squared as effect size), through a multivariate test, was 99% (0.33) for time, 68% (0.64) for group, and 98% (0.25) for time*group (supplement 1).

Results

Figure1 shows the trial profile and patient flowchart, based on the CONSORT-statement (http://www.consort-statement.org) guideline. A total of 20 patients were screened and divided into two groups of patients with refractory GERD. The mean age in the midodrine group (36.30 ± 11.44) and placebo group (37.50 ± 10.30) were not significantly different (P = 0.808). The mean duration of GERD symptoms was not significantly different between the groups (midodrine: 49.10 ± 46.90, Placebo: 50.80 ± 74.27 months; P = 0.952). Also, BMI, gender, ethnicity, smoking status, sign and symptom, endoscopic and pathologic findings were the same in either arm of the study. The demographic and clinical information of both groups are shown in Table 1.

Table 2 shows the detailed comparison of the measured scores between the groups over the time. Repeated measure ANOVA showed that there was a significant interaction effect between the group and time on all of the measured scores (Table 2). Although all the markers in the midodrine group had significant changes over the time, the placebo group did not show any significant changes. These changes with related confidence interval in each time point are shown in Figure 2. Visual severity score in midodrine shows a U shape change during the time. It had a significant decrease in week 2 (P = 0.001) and week 4 (P = 0.001) compared with the baseline and a significant increase in week 6 (P = 0.05). Emotional score, sleep score, food score, physical score, vitality score, and total QoL in Midodrine group showed an inverse U shape changes during the time. All of them had a significant increase in week 2 and week 4 compared with the baseline and a significant decrease in week 6.

In week 2, visual severity score in midodrine group had higher mean than placebo group (P = 0.004); and emotional score (P = 0.020), food score (P = 0.045), vitality score (P = 0.010), and total QoL (P = 0.040) in midodrine group had significantly higher mean than placebo group. In week 4, the same pattern in differences between the groups remained statistically significant only for visual severity score (P = 0.002), Emotional score (P = 0.049), and Vitality score (P = 0.028). There were not any significant differences between the groups in week 6. No significant adverse effect/change in blood pressure was observed in either case or control groups.

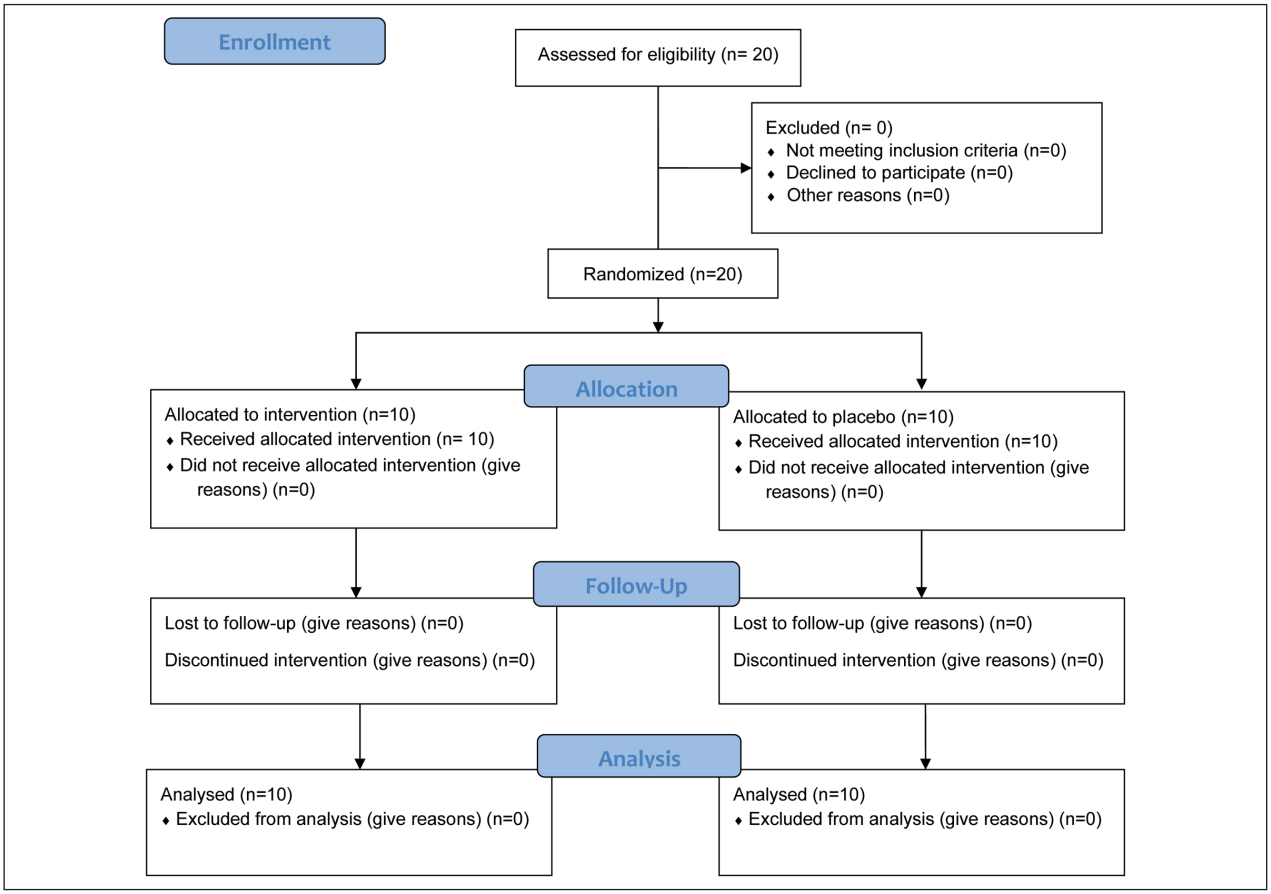

Figure 1. CONSORT Flow Diagram

Table 1. Demographic and clinical information of patients in midodrine and placebo groups

		Midordine	Placebo	P-value
Age		36.30±11.44	37.50±10.30	0.808
BMI		24.46±3.62	24.34±4.31	0.946
Duration of disease		49.10±46.90	50.80±74.27	0.952
Gender	Female	5(50)	6(60)	0.999
	Male	5(50)	4(40)	
Ethnicity	White	9(90)	10(100)	0.999
	Black	1(10)	0(0)	
Ex-Smoker	Yes	1(10)	1(10)	0.999
	No	9(90)	9(90)	
Gesture at symptom	Upright	2(20)	4(40)	0.536
	Supine	5(50)	2(20)	
	Both	3(30)	4(40)	
Endoscopic findings	Normal	5(50)	6(60)	0.999
	GERD	5(50)	4(40)	
Pathologic finding	Reflux esophagitis	8(80)	6(60)	0.628
	Reflux and Hpylori	2(20)	4(40)	

Table 2. Comparison of the measured scores according to QOLRAD[1] questionnaire between the groups over the study time

		Baseline	Week 2	Week 4	Week 6	P_{Time}	P_{Group}	$P_{Time*Group}$
Visual severity score	Midodrine	7.60±1.71a	4.70±2.58a	3.90±2.46a	6.60±2.11a	**0.001**	**0.011**	**0.001**
	Placebo	8.60±1.77	8.20±2.14	8.00±2.62	8.30±2.21			
	P	0.216	**0.004**	**0.002**	0.096			
Emotional score	Midodrine	19.90±7.89ab	27.80±9.50ac	27.20±9.50bd	20.10±8.53cd	**0.001**	0.138	**0.005**
	Placebo	17.11±8.76	18.44±6.65	18.44±8.32	18.33±9.27			
	P	0.482	**0.020**	**0.049**	0.671			
Sleep score	Midodrine	21.00±6.54ab	25.80±8.89ac	26.20±7.37bd	21.70±5.90cd	**0.003**	0.202	**0.007**
	Placebo	19.66±8.67	19.22±5.80	19.66±8.45	18.77±7.71			
	P	0.579	0.052	0.090	0.364			
Food score	Midodrine	19.60±8.11ab	27.40±9.14ac	26.20±8.70bd	19.00±8.31cd	**0.001**	0.442	**0.001**
	Placebo	20.55±7.89	20.11±5.96	21.00±7.82	19.55±7.65			
	P	0.821	**0.045**	0.191	0.882			
Physical score	Midodrine	21.00±6.35ab	26.50±8.56ac	27.20±8.23bd	20.80±7.91cd	**0.001**	0.696	**0.001**
	Placebo	23.77±5.56	22.66±5.72	22.88±6.11	21.33±6.18			
	P	0.281	0.254	0.217	0.873			
Vitality score	Midodrine	10.00±3.52ab	14.70±4.39ac	15.60±4.59bd	9.80±4.04cd	**0.001**	0.159	**0.002**
	Placebo	10.00±4.35	9.88±3.62	10.44±4.74	9.44±4.36			
	P	0.823	**0.01**	**0.028**	0.856			
Total quality of life score	Midodrine	91.50±30.22ab	122.30±39.67ac	122.40±36.93bd	91.40±32.18cd	**0.001**	0.266	**0.001**
	Placebo	91.22±32.01	90.33±25.38	92.44±32.65	87.44±33.02			
	P	0.936	**0.040**	0.080	0.795			

Within rows, the same lower letter indicates the significant difference between two time periods
[1] QOLRAD: Quality of Life in Reflux and Dyspepsia questionnaire

Discussion

Gastroesophageal reflux disease (GERD) is a prevalent disease, which can adversely affect several aspects of patients' lives including their productivity at work (23).

Although proton pump inhibitors (PPIs) are the mainstay in GERD medical management, around one third of these patients do not respond to PPIs once daily. Significant proportion of these patients show improvement in their symptoms after increasing the standard dose of PPIs twice (BID) daily. As a result, the use of PPIs in BID dosages is a common practice and standard of care in GERD patients who stay refractory to PPIs once daily. In this investigation, we studied this group of patients with refractory GERD who remained symptomatic despite being treated with PPI BID (8, 24-28).

Although gastric acid is the principal noxious agent in GERD, increased episodes of transient lower esophageal relaxations (TLESRs) has been considered as one of the major pathogenetic factors in GERD. TLESR occurs as a physiologic response to gastric distension through vagal stimulation in healthy individuals. Increased episodes of TLESRs accounts for 65% of reflux episodes. Based on these findings TLESR has been considered as an attractive target for treating GERD. Gamma-aminobutyric acid (GABA) receptor type B agonist such as baclofen and lesogaberan have been shown to reduce TLESRs and decrease acid reflux episodes and increase LES pressure in several clinical trials. These drugs have major side effects including somnolence, fatigue, and dizziness, which some patients cannot tolerate (14, 15, 29-40). Acotiamide has been recently introduced as an option for treating refractory GERD, which reduces TLESRs and enhances

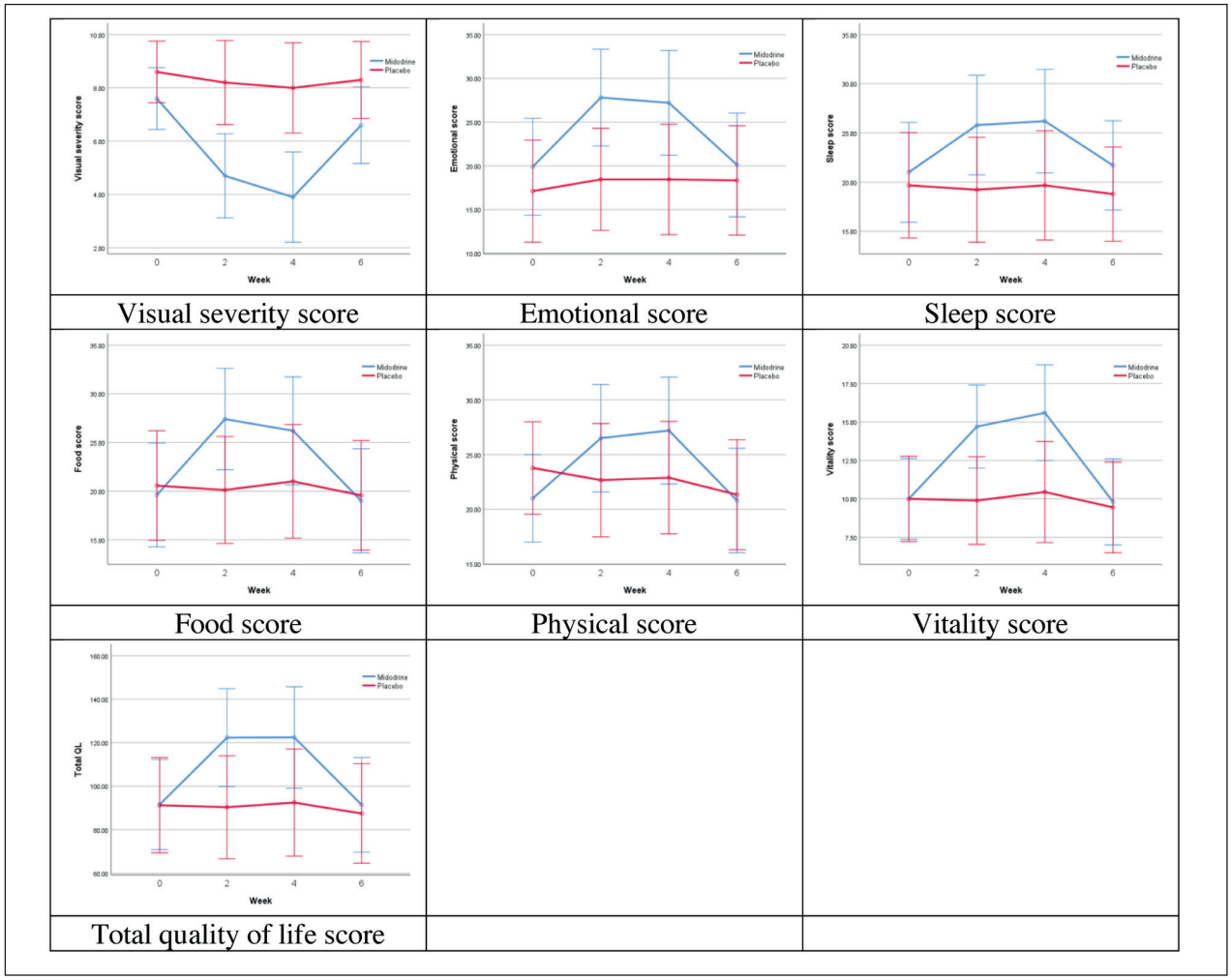

Figure 2. Comparison of the measured scores according to Quality of Life in Reflux and Dyspepsia questionnaire (QOLRAD) questionnaire between the groups over the study time

esophageal motility. However, its efficacy in improving symptom of refractory GERD remains to be elusive (16). Midodrine, an alpha-1 adrenergic agonist, in clinical practice is mainly used to treat orthostatic hypotension and hepatorenal syndrome. It is metabolized to its active metabolite, desglymidodrine, with peak blood levels reaching within 30-60 minutes after oral intake. The drug has good oral bioavailability and safe side effect profile (41, 42). In this double-blind, randomized controlled study on patients with refractory GERD midodrine 5mg 30 minutes before meal significantly improved symptom severity based on visual scorings. The beneficial effect increased during treatment for four weeks, but was aborted two weeks after discontinuation of treatment. Through QOLGAD, the standardized and validated questionnaire for measuring the QoL in patients suffering from GERD, we were able to show a significant improvement in both overall QoL score and all other aspects after midodrine usage. As with severity score, changes in QOLGAD parameters had a time pattern. The scores improved in the second and fourth week while being on midodrine and then decreased to baseline two weeks after discontinuation of midodrine. No adverse event was reported. For the first time in this study, we showed that midodrine could be useful in managing refractory GERD.

Maybe, some patients suffering from functional heartburn rather than acid-based reflux. But at this

group, midodrine had efficacy and in this group, they also benefit from taking medication. Finally, Even in this group using midodrine is better in compare to use Common treatment such as anti-depressant.

This study used rigorous inclusion criteria to avoid the effect of confounders. Since this is a pilot study, it has several limitations. Small sample size (supplement 1) and lack of pH-metric and impedance results are amongst the major weaknesses of our study. However, this is the first study on the effect of midodrine in refractory GERD patients and in future studies these limitations should be acknowledged.

In conclusion, midodrine before a meal could be effective in alleviating symptoms and improving QoL in patients with refractory GERD. We recommend larger trials with adequate sample size; in addition to pH-metric and impedance to unravel the probable midodrine mechanism of action.

Key Points

- A proportion of patients with gastroesophageal disease are refractory to potent anti-acid agents including proton pump inhibitors. This study evaluated the role of midodrine in the management of refractory patients.
- Use of midodrine lead to significant improvement in both severity of symptoms and quality of life of refractory reflux patients.
- Midodrine could be an upcoming safe medication in the management of reflux disease in refractory patient.

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Compliance with ethical standards: The study was approved by the local Ethics Committee of Shiraz University of Medical Sciences, code number CT-P-92-6683.

Author contributions: Lankarani KB, Sivandzadeh GH R: conception, design, editing, and decision to publish. Sivandzadeh GH R: data collection, assistance with data analysis, interpretation of the data, manuscript writing, editing, and decision to publish. Nejati M, Niknam R, Taghavi AR, and Ejtehadi F, Alizadeh NM, Moini M and Anbardar MH: Case selection and decision to publish. Peymani P, Zare M: Design, analysis, interpretation of the data, and decision to publish.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Amino acids and fatty acids in patients with beta thalassemia major

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Summary. *Background:* Oxidative damage and increasing of lipid peroxidation are caused by chronic iron overload in patients with beta thalassemia major. Fatty acids are important structural elements for palmitoylation of membrane proteins which constitute a great part of natural membranes. Oxidative damages caused by reactive oxygen derives in thalassemic erythrocytes can be determined with lipid peroxidation, protein oxidation, and antioxidant system elements. The aim of study was to evaluate the relationship between amino acid and fatty acid levels with iron overload and antioxidant enzymes in beta thalassemia major. *Methods:* A total 40 patients with beta thalassemia major with regular blood transfusion and chelating agents were included in the study. The levels of serum amino acid, fatty acid, ferritin, antioxidant enzymes and malondialdehyde were measured. *Results:* Only C16- palmitoyl level was found significantly low in patients, other fatty acids and amino acids were in normal range. There were lower malondialdehyde and ferritin levels in patients with low C-16 palmitoyl level ($p<0.05$). *Conclusions:* The high levels of ferritin and malondialdehyde in the patients with low C16-palmitoyl levels might be caused by this fatty acid's preventative effect on oxidative stress. (www.actabiomedica.it)

Key words: amino acids, antioxidants, fatty acids, ferritin, thalassemia major

Introduction

Many recent studies about oxidative stress and antioxidant system in patients with beta thalassemia major (β-TM) show decreasing in antioxidant levels and increasing in the lipid peroxidation of erythrocyte membranes. The oxidative stress causes are the unpaired excess alpha chains, non-Hb iron and low levels of intracellular hemoglobin (1). Since the cross-reactivity between free radicals and molecules that including unsaturated fat and sulphate, proteins that have amino acids such as phenylalanine, tyrosine, tryptophan, histidine, and methionine are easily affected by free radicals (2). Fatty acids are important structural elements for palmitoylation of membrane proteins which constitute a great part of natural membranes (3). It has been argued that fatty acids are metabolic energy sources, play an important role in cell homeostasis, affects immune system, and that some of them show antimicrobial and anticancer activity (4). The aim of study was to evaluate the relationship between amino acid and fatty acid levels with iron overload and oxidative stress in β-TM.

Methods

A total 40 patients with β-TM aged 7–30 years were included in this study. Twenty-two of 40 had splenectomy. Blood samples were taken as late as pos-

sible, at least 3-4 weeks after the last transfusion. The patients did not take any medications for at least 1 week prior to blood sample collections except iron chelating agents. All patients have been treated with regular blood transfusion (15 cc/kg per month) and chelating agents (30 mg/kg/day deferasirox). The blood samples of the patients were taken for the levels of serum ferritin, amino acids, fatty acids, malondialdehyde (MDA), catalase (CAT), glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD). As amino acids; valine, leucine/isoleucine, methionine, phenylalanine, tyrosine, aspartate, glutamate, argininosuccinate, ornithine, citrulline, glycine, alanine, arginine, phenylalanine/tyrosine and as free fatty acids; free carnitine, C2-acetyl, C3-propionyl, C4-butyryl,C5:1-tiglyl, C5-isovaleryl, C4-OH-3-OH-butyryl, C6-hexanoyl, C5-OH-3-OH-isovaleryl, C8-octanoyl, C10-decanoyl, C5-DC-glutaryl, C12-dodecanoyl, methyglutaryl, C14:1, C14-myristoyl, C14-OH-3-OH-myristoyl, C16:1-palmitoleil, C16-palmitoyl, C18:1-OH-3-OH-oleoyl, C16-OH-3-OH-palmitoyl, C18:1-oleoyl, C18-stearoyl, C16:1-OH-3-OH-palmitoleyl levels were measured by using electrospray tandem mass spectrometry method (5). Ferritin levels were studied at the same day and the samples for the antioxidant study stored at -80°C and analyzed by spectrophotometric method. The SOD level was measured by the method described by Williams al (6). Measurement of GSH-Px activity was based on the method of Paglia and Valentine (7). The activity of catalase enzyme was measured by the Aebi method (8). MDA levels were measured by the double heating method of Draper and Hadley (9).

Serum AST and ALT estimation was done by semi auto analyser. Serum level of AST >40 IU/L and ALT >38 IU/L were considered abnormal (10).

Exclusion criteria were samples with recorded hemolysis, and lypemia.

Statistical Analysis: SPSS 15.0 packaged software was used for the statistical evaluations for the statistical analysis, Mann Whitney U test was used to compare the data and Pearson correlation coefficient was computed to show the correlation between variables. This study was carried out with the permission of Ethics Committee of Medical School of Suleyman Demirel University and consents of the patients' relatives within The Helsinki Rules.

Results

The study involved 40 patients (62.5% female), with the mean age of 18.58±5.7 years. 10 patients were older than 20 years, 22 patients were between 15-19 years old, and 8 patients were younger than 14 years.

Mean serum ferritin was 4533±2116 (577-10741) ng/mL. Mean AST was 84.90±9.92 IU/L and mean ALT was 108.32±15.97I U/L which were higher than normal value. Serum AST and ALT levels were found to be above three times the normal range in 27.5% and 20% of patients, respectively. 13 (32.5%) of the patients' serum ferritin levels were between 2000 and 4000 ng/mL, 12 (30%) of the patients' serum ferritin levels between 4000 and 6000 ng/mL and 10 (25%) of the children had even higher serum ferritin levels (>6000 ng/mL) in spite of the chelation. Only 5 (12.5%) patients maintained serum ferritin levels <2000 ng/ml. Serum liver enzymes at various levels of serum ferritin levels were as shown in Table 1. The statistically significant difference in AST and ALT was observed once the serum ferritin crossed level of 4000 ng/mL ($p < 0.05$).

As liver enzymes were analyzed at different serum ferritin levels, simultaneously rising as the serum ferritin was increasing. A steep rise in liver enzyme was noticed after the level of 2000 ng/mL as shown in Figure 1.

As fatty acids, free carnitine, C2-acetyl, C3-propionyl, C16-palmitoyl were found as low in 1 (2.5%), 3 (7.5%), 8 (20%) patients; respectively, C18:1-oleoyl was found as high in 2 patients (5%). Other fatty acid levels were found in normal range. The mean value of MDA of 14 patients with low C-16 palmitoyl levels was 155.47±16 nmol/gr, while the mean value of MDA of 26 patients with normal C-16 palmitoyl levels was 129.38±11 nmol/gr. This MDA difference between low and normal C-16 palmitoyl levels is statistically significant ($p<0.05$). While the mean value of ferritin levels of 14 patients with low levels of C-16 palmitoyl was 5595.85±517 ng/dL, the mean value of ferritin levels of 26 patients with normal C-16 palmitoyl levels was 3993.0±390 ng/dL. This difference in ferritin levels is statistically significant ($p<0.05$) (Table 2).

The relationship between ferritin and MDA with antioxidant enzyme levels was evaluated. Although SOD level increases and MDA, GSH-Px, catalase levels decrease with ferritin increases, there was no sig-

Table 1. Liver enzymes at different serum ferritin levels

Ferritin (ng/mL)	n	AST (IU/L) (mean ± SD)	p	ALT (IU/L) (mean ± SD)	p
<2000	5	44.40 ± 27.01	>0.05	36.80 ± 20.14	>0.05
2000-4000	13	58.38 ± 30.06	>0.05	65.15 ± 35.42	>0.05
4000-6000	12	92.08 ± 50.83	**0.02**	118.33 ± 98.89	**0.02**
>6000	10	131.00 ± 89.57	**0.02**	188.20 ± 132.87	**0.01**

n: Number of cases; SD: Standard deviation

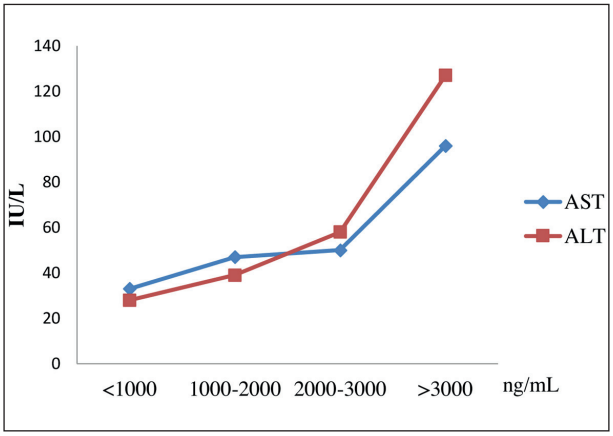

Figure 1. Trend of liver enzymes with rising serum ferritin

nificant correlation between ferritin and antioxidants (Table 2).

Correlation between ferritin and amino acid levels were evaluated. It was found only weak negative correlation between ferritin and glutamate. There was no significant correlation between ferritin and other amino acid levels. We also evaluated the correlation between ferritin and fatty acid levels. As a consequence, we found a weak negative correlation among ferritin, carnitine, C6-hexanoyl, C8, C12-dodecanoyl, and C14.

Discussion

Liver is the earliest organ affected by iron overload in thalassemia children and serum AST and ALT are raised due to peroxidative injury and direct toxic effect of iron on liver cells. In present study the serum ferritin concentration was very high in β-thalassemic children inspite of chelation therapy. Like other chronic diseases requiring long-life treatment, adherence to treatment is a major concern for β-TM patients. Poor adherence

Table 2. Comparison of C16-palmitoyl values with MDA, level of antioxidant enzymes and ferritin

C16-palmitoyl	n	MDA $\overline{X} \pm S_{\overline{x}}$	CAT $\overline{X} \pm S_{\overline{x}}$	GSH-Px $\overline{X} \pm S_{\overline{x}}$	SOD $\overline{X} \pm S_{\overline{x}}$	Ferritin $\overline{X} \pm S_{\overline{x}}$
Normal	26	129.38±11	396.82±61	59.75±2	1956.73±87	3993.00±390
Low	14	155.47±16	322.69±65	52.24±2	1866.22±106	5595.85±517
p*		**0.02**	0.70	0.05	0.61	**0.01**

*Mann Whitney U
n: Number of cases, $\overline{X}$: Arithmetic mean, $S_{\overline{x}}$: Standard error mean

MDA: Malondialdehyde, CAT: Catalase, GSH-Px: Glutathione Peroxidase, SOD: Superoxide Dismutase

Table 3. Correlations between ferritin, MDA, CAT, GSH-Px, SOD

	MDA (r;p)	CAT (r;p)	GSH-Px (r;p)	SOD (r;p)
Ferritin	-0.012;0.943	-0.106;0.515	-0.014;0.933	**0.274;0.092**
MDA		0.036;0.830	0.147;0.380	**-0.060;0.726**
CAT			-0.006;0.971	**0.203;0.216**
GSH-Px				**0.062;0.707**

r: Pearson's correlation coefficient
MDA: Malondialdehyde, CAT: Catalase, GSH-Px: Glutathione Peroxidase, SOD: Superoxide Dismutase

remains a prevalent and persistent problem in these patients, with the reported rates ranging from 30 to 80 percent (11,12). It has been revealed that poor adherence to therapeutic regimen is associated with poor clinical outcomes including deranged liver functions. The study showed that AST and ALT were raised significantly ($p<0.05$) and continue to rise as ferritin crosses 2000 ng/ml. These findings are in agreement with other previous studies who reported that serum ferritin increases liver enzymes also increases (13-16).

Fatty acids are important structural elements of natural membranes and constitute a great part of these membranes. Oxidative damages caused by reactive oxygen derivates in thalassemic erythrocytes can be determined with lipid peroxidation, protein oxidation, and antioxidant immune system elements. Increasing of lipid peroxidation occurs with increasing of MDA (3,4,17,18). The MDA levels of patients have low fatty acid levels were evaluated and found only correlation between C16-palmitoyl. The mean value of MDA levels of the patients with low C16-palmitoyl levels was higher than that of the patients with normal C16-palmitoyl levels ($p<0.05$). This decreasing in C16-palmitoyl level may demonstrate tissue damage caused by free radicals. There has been no published study about free fatty acid levels of patients with thalassemia major.

There are various studies about antioxidant enzyme levels (18-22). As compensatory reply to lipid peroxidation, some studies show increase in antioxidant enzyme (SOD, GSH-Px, CAT) levels while some other studies show decrease in these enzyme levels (23,24). This result can be explained by the increasing of oxidative stress (25) or insufficient chelation treatment causes iron overload (26). In this study, erythrocyte GSH-Px, SOD and CAT enzyme activities of patients with low and normal serum fatty acid and amino acid levels were compared and there was no statistically significant difference.

Increased oxidative degradation caused by iron overload is important in pathogenesis of thalassemia. Increment of lipid peroxidation in chronic iron overload was shown in experimental animals and organs of patients with thalassemia. Livrea et al. have detected the positive correlation between serum ferritin level and MDA level in 42 patients with β-TM and have 1.866±996 ng/mL mean level of ferritin (27). In a similar study, Naithani et al. have detected serum ferritin level as 3.709±1.625 ng/mL in 50 patients with β-TM and observed negative correlation between serum ferritin level and GSH-Px level (25). There was no significant correlation between serum ferritin level and antioxidant enzyme levels (CAT, SOD, GSH-Px) with MDA was detected in the patients with β-TM who had 4.554±2.095 ng/mL of serum ferritin levels. Our findings give rise to thought that there might be other factors play role in the increment of oxidative stress and peroxidative tissue damage by free radical production than iron overload.

Vander Jagt DJ et al. reported a decrement in concentration of plasma amino acid and increment in urinary amino acid loss and they pointed out that this might contribute to the decrease in growth rate in children with sickle cell anemia (28). In a similar study was showed a significant decrease in isoleucine, phenylalanine, tyrosine, taurine, glutamine levels in children with thalassemia major comparing to the control group (29). In our study, as essential amino acids, methionine level was found as low in 2 patients and as

non-essential amino acids, glycine level was found as low in 4 patients; other amino acid levels were found in normal range. No significant relationship between MDA, antioxidant enzymes and ferritin levels of the patients with low normal levels of methionine and glycine was found.

Palmitoylation is important especially in stabilization of cell membranes. The most important fatty acids for palmitoylation are 16 carbon saturated fatty acids (4). S-palmitoylation involves the attachment of a 16-carbon long fatty acid chain to the cysteine residues of proteins (30). In our study, C16-palmitoyl levels of 14 patients were found as low. Ferritin and MDA levels of the patients with low C16-palmitoyl levels were higher than those of the patients with normal C16-palmitoyl levels. These findings can be associated with free radical production in which iron overload plays role and that results in peroxidative tissue damage. In addition, high levels of ferritin and MDA in the patients with low C16-palmitoyl levels might be because of this fatty acid's preventative effect on oxidative stress.

In conclusion, oxidative damage and increase in lipid peroxidation are caused by chronic iron overload in patients with β-TM. The most significant indicator of this damage is the increase in MDA levels. Membrane lipids and proteins show hypersensitivity to peroxidative damage caused by iron. In our study, the most significant finding was the decrease in C16-palmitoyl level whereas no significant result about other amino acid and fatty acid levels was found. In patients with low C16-palmitoyl levels, high ferritin and malondialdehyde levels may be due to the inhibitory effect of this fatty acid on oxidative stress.

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Author contribution: DC contributed to the conception and design of this study; TK, and YSK performed the statistical analysis and drafted the manuscript; TK, HD and YSK collected data; AB analyzed data; TK and DC wrote the manuscript; DC, ARO, and MA critically reviewed the manuscript and supervised the whole study process. All authors read and approved the final manuscript.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Original article

Enhancing self-resources in patients with chronic diseases: development and initial validation of the Disease and Care Management Score

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Summary. *Introduction:* Despite the importance of the assessment in the primary care of the self-resources among patients with chronic diseases, there is not available a measurement that allows this kind of comprehensive assessment. For this reason, the aim of this study was to develop a multi-dimensional score to determine the level of self-resources in chronic patients, describing its initial validation through face and content validity. The developed score was labelled as Disease and Care Management Score. *Methods:* We performed a methodological study, encompassing two main phases. The first phase was aimed to develop the Disease and Care Management score, choosing the most suitable measurement to assess each pre-identified determinant of wellbeing in chronic patients. The second phase was aimed to determine the Disease and Care Management score face and content validity through the views of 20 experts. *Results:* Disease and Care Management score shows evidence of face and content validity. All the obtained quantitative content validity indices (i.e. Content Validity Ratio, Content Validity Indices) were higher than 0,70, showing the pertinence and the adequacy of each pre-identified measure to compute Disease and Care Management score. *Conclusion:* Disease and Care Management score has the potential of addressing the health coaching interventions in primary care for chronic patients. Future research should show its predictive performance, as well as the cut-off to discriminate patients. (www.actabiomedica.it)

Key words: chronic care, coaching, education, wellbeing, self-resources, primary care

Introduction

Chronic diseases require continuous treatments over time, which could last from years to decades (1). Currently, caring for people with chronic diseases involves 70-80% of the economic resources of healthcare with slight differences among Countries (2). The increased epidemiology of chronic diseases is strictly linked to the population ageing, as well as the improvements in the healthcare delivery, and the enhancements of the economic and social conditions (3). Accordingly, the population over 65 among the European Countries has raised from roughly the 10% in 1960s to the 19% in 2015, being expected to increase towards the 30% by 2060 (4). This scenario comes as a result of considerable progress in life expectancy, and opens new areas, which are worthy of investigation. Thus, research should address the issues underpinning the improvement of quality of life in elderly as well as the life expectancy (5). Accordingly, it is estimated that roughly 50 million of Europeans have two or more chronic diseases, where these diseases tend to correspond with an increased aging (4). As per Italy, roughly 39% of the population is affected by a chronic disease, where almost the 45% of those patients are aged over sixty-five (5, 6). More precisely, diabetes, cardiovascular diseases, stroke, COPD, cancers and dementias affect the 18% of the Italian population with a chronic disease (6).

The traditional hospital-based approaches of healthcare delivery seem to be unsuitable for addressing the needs of patients with chronic diseases (2). In fact, the treatment of chronic diseases does not purpose to healing patients, but it is mainly aimed to enhance patients' self-resources in managing their condition to achieve an improvement of their functional status, adherence to treatments, and an increased quality of life (7). Accordingly, the appropriate management of care pathways is pivotal, and it should be proactive, multidimensional, patient-centered, and globally aimed to empower the patients (7, 8). Further, chronic disease management interventions should address the possible inequalities in the accesses for primary care, such as in the case of some minority groups (9).

So far, healthcare systems are mainly organized to treat acute and episodic diseases (10). However, the Chronic Care Model (CCM), developed in the late 1990s, provides a framework to face with the current burden of chronic diseases (11). Properly, CCM was designed to engage patients and improve their health outcomes by changing the routine delivery of ambulatory care, shifting the paradigm of care from being reactive towards the characteristics of proactivity, planning population-based interventions where possible (12). Some authors have also identified in the CCM an opportunity to re-organize care, emphasizing the need of a coordination of the care path to allow a continuum between the various healthcare systems consulted by chronic patients (11). Many authors emphasized the importance of the patients' stratification to allow the correct identification of specific pathways (7, 13).

However, the current possibilities to helpfully stratify the chronic population led to a high heterogeneous scenario. For instance, patients could be stratified using the diagnosis taxonomy, their behaviors or lifestyles, their adherence to treatments and follow-ups (14). The choice to select patients for specific educational paths is currently undermined by the unavailability of a measure that encompasses the main different characteristics (i.e. determinants of wellbeing) described in chronic patients, which are dietary habits, physical resources, adherence, psychological status, ability to perform daily activities, self-care, and the overall perceived quality of life (15). A comprehensive score of the above-mentioned determinants could be useful to select patients for specific educational or support interventions, using an evidence-based approach. Further, we can argue that, theoretically, a comprehensive score could also present some important predictive characteristics towards the decline of clinical conditions, re-hospitalizations, and even mortality. For all these reasons, this study was aimed to develop a multi-dimensional score to identify the comprehensive health status of patients with chronic diseases, providing its initial validation through face and content validity. The developed measure was labelled as "Disease & Care Management Score" (D&CM).

Methods

This was a multi-phase and methodological study, encompassing two phases: phase one referred to the development of the D&CM score, while phase two referred to its face and content validity.

Development of D&CM score

The development of D&CM score was mainly based on a previous description of individual and social determinants of health in population with chronic diseases (15). Then, these determinants were categorized into dietary habits, physical activity levels, adherence to treatments, psychological burden, and overall functional status. A panel of three expert authors (AP, RC, AC) in chronic diseases operationalized the measures needed to assess each health determinants. Accordingly, authors identified the following valid and reliable measures for each determinant, that subsequently need to be scored in a unique measure to identify a comprehensive health status score (D&CM score).

Dietary habits and physical activities were detected using a scale developed by the research center of the Mario Negri Institute (16). This self-report scale encompassed 12 items to explore dietary habits, and three items to explore physical activities. Conversely, we selected the beliefs about medicines questionnaire (BMQ) as a proxy assessment for patients' adherence (17). In fact, some authors used BMQ to explore patients' adherence, because there is a strong relationship between beliefs about medicines and the actual adher-

ence to pharmacological treatments (18). Psychological burden was explored for one of its major problematic aspect, i.e. depression. For this reason, we chose the Hamilton depression rating scale (HAMD) to intercept patients' depression (19). Finally, Karnofsky's scale correlates with physical functioning, such as walking and stair climbing, and it has predictive validity for poor prognosis (20). All the above mentioned measures of health determinants were kept into account to develop the initial version of D&CM score. In other words, D&CM score embodied all the measurements required to assess dietary habits and physical activities, BMQ, HAMD and Karnofsky into a single score.

D&CM scoring procedure

All the scores coming from the different used scales (raw scores) were standardized in a comprehensive score ranging from one to four. The standardization of the different scores coming from the original scales was aimed to adjust each values measured on different metrics to a common measure. We used formula of Z-score through 'standardization of normal distribution', as described by several authors (21). Overall, D&CM scoring procedure is available using an ad hoc software developed by GPI Group (AC).

Face and content validity

Once terminated the initial choice for selecting scales aimed to assess each single health determinant, we performed the face and content validity study for the overall D&CM score. Content validity refers to the methodology developed in the 1970s by Lawshe (22), being aimed to detect the level of the agreement among expert raters in defining the pertinence of each measure in relation to the objective of the overall measurement (identifying a comprehensive score to explore health status in patients with chronic conditions). More precisely, content validity encompasses the quantitative assess of raters using the Content Validity Ratio (CVR), and the Content Validity Index for item and scale level (I-CVIs and S-CVI). CVR could potentially range between -1 (perfect disagreement among panellists) and +1 (perfect agreement among panellists), while I-CVIs and S-CVI range between 0 (no content defined as valid) and +1 (content totally judged as valid). As per the CVR critical values (i.e. the lowest level of CVR such that the level of agreement was greater than 50%), the recent literature proposed a revisiting of the originally-developed critical values, considering that the CVR critical values have been originally determined by the normal approximation to the binomial distribution applied to the panel sizes encompassing less than 13 panellists (23). More precisely, the normal approximation to binomial has been caused concerns on CVR critical values (only when panel size is lower to 13 participants due to for larger sizing no approximation was used), as the critical values determined with this approach seem to be inferior to the ones determined using the exact binomial probabilities (24). For this reason, the recent proposed CVR critical values seems to be more prudent in defining the values expressing a level of agreement among panellists higher than 50% for a given α (i.e. type I error probability, which is 0,05 using a one-tailed test), even when panel size encompasses less than 13 panellists (24). Conversely, face validity explored experts' understanding of each measure, and their views about eventual amendments to improve the overall content of D&CM score (25). Both face/content validity could have brought some amendments to the initial authors' choice in operationalizing the individual and social determinants of health in chronic population, using the above-described scales.

Further, face/content validity implicitly requires the selection of a panel of experts to provide their judgments on the proposed items aimed to measures the content area it is expected to measure with the D&CM score (i.e. a multi-dimensional score to identify the comprehensive health status of patients with chronic diseases). Overall, the panelists' answering closely determines this kind of validity, and for this reason the selection of the panelists is pivotal to ensure rigor. Despite the determination of the number of panelists is generally partly arbitrary, when the number of panelists increases, the probability of chance agreement decreases (26). Accordingly, higher is the number of panelists, higher is the difficulty in find agreement. The first recommendations to determine the choice of the experts were proposed in the 1980s (27), being criticized and discussed during the last two decades (28, 29). Overall, the literature suggests to invite

the panelists using a declared rationale of selections, which represents the pros and cons of the overall content validity process.

Having said that, we decided to invite a relatively large number of panelists (n=20), decreasing the implicit possibility of achieving high rates of agreement, but increasing the caution in determining the content validity in relation to the aim of D&CM score. In this study, the evaluated items were related to previously validated scales with the purpose to embody these different measures into a single score. Further, the selection of panelists was performed inviting them from a list of educators involved in post-graduate courses on chronic management for both the continuing medical education program (CME) and university-level courses on chronic management in nursing (bachelor) and/or medicine (MD program) courses. More precisely, the selection of the panelists coming from the list of educators was guided by the following inclusion criteria: (a) medical degree (MD) or Master of Science (MSc) degree; (b) five years of minimum working experience with chronic patients (excluding internships or similar educational trainings); (c) active involvement as educators on topics related to the caring for chronic patients (e.g. professors, tutor, mentor). Overall, content validity is generally considered as the initial step of a complex validation process that often requires more inferentially robust analysis to determine construct validity, such as multivariate latent variable modeling (e.g. exploratory factor analysis, exploratory structural equation modelling).

Ethical considerations

This study did not involve patients. The authors planned the designing, conducting, recording and reporting of the study in a consistent way with the international ethical and scientific quality standards, indicated by Good Clinical Practice (GCP) and standard operating procedures (SOPs). All the involved experts were informed on the study aim.

Statistical analysis

Socio-demographics of the involved experts were represented using descriptive statistics. CVR was computed as follow: CVR=(N*e* - N/2)/(N/2), in which the N*e* is the number of raters indicating "essential" and N is the total number of raters. It could varies between +1 and -1, where higher score indicates higher agreement among raters. The interpretation of CVR was performed comparing the observed CVR coming from the panelists' answering and the critical CVR recently proposed using the discrete binomial calculations, given the discrete nature of the variables used to compute CVR (24). To obtain I-CVIs, we calculated the number of those judging the measurement as relevant (i.e. ratings ≥ 3) divided by the number of content experts. Thus, I-CVIs expressed the proportion of agreement on the relevancy of each measure, where the index could range between zero and one (23). Furthermore, S-CVI was defined as the proportion of total items judged content validity (23), computing the mean of each obtained I-CVIs.

Results

The enrolled experts involved for the content and face validity were 20, being selected considering the pre-defined inclusion criteria aimed to guide their selection. They were mainly males (n=11; 55%), physicians (n=11; 55%), their median of age was equal to 43,7 years (IQR=8,3 years) with a median of 19,6 years of experience (IQR=7,9 years) (Table 1).

As Table 2 shows, the obtained CVRs were higher than 0.70, as well as the I-CVIs and each S-CVI. Thus, no need of amendments occurred by this step of validation. Further, the narrative analysis on the free-text comments for each scale selected to compute D&CM

Table 1. Characteristics of the experts (n = 20)

		N	%
Gender	Male	11	55
	Female	9	45
Profession	Physician	11	55
	Nurse	9	45
		Median	IQR
Age		43,7	8,3
Years of experience		19,6	7,9

Legend: IQR = interquartile range

Table 2. Content validity scores

Expert panellists (n = 20)	N*e*	CVR	Interpretation	I-CVIs	Interpretation	S-CVI	Total Score S-CVI
Scale on dietary habits							
Item 1	19	0,9	Relevant	0,9			
Item 2	18	0,8	Relevant	0,85			
Item 3	18	0,8	Relevant	0,9			
Item 4	19	0,9	Relevant	0,9			
Item 5	20	1	Relevant	0,95			
Item 6	18	0,8	Relevant	0,9	Pertinent	0,9	
Item 7	18	0,8	Relevant	0,85			
Item 8	19	0,9	Relevant	0,95			
Item 9	20	1	Relevant	1			
Item 10	17	0,7	Relevant	0,8			
Item 11	20	1	Relevant	0,95			
Item 12	19	0,9	Relevant	0,85			
Scale on physical activities							0,90
item 1	19	0,9	Relevant	0,88			
item 2	20	1	Relevant	0,95	Pertinent	0,91	
item 3	18	0,8	Relevant	0,85			
Beliefs about medicines questionnaire							
item 1	18	0,8	Relevant	0,9			
item 2	18	0,8	Relevant	0,85			
item 3	19	0,9	Relevant	0,95			
item 4	20	1	Relevant	1			
item 5	17	0,7	Relevant	0,8			
item 6	20	1	Relevant	0,95			
item 7	18	0,8	Relevant	0,85	Pertinent	0,91	
item 8	19	0,9	Relevant	0,88			
item 9	20	1	Relevant	0,95			
item 10	18	0,8	Relevant	0,95			
item 11	18	0,8	Relevant	0,88			
Hamilton depression rating scale							
Item 1	20	1	Relevant	0,9			
Item 2	17	0,7	Relevant	0,85			
Item 3	20	1	Relevant	0,95			
Item 4	18	0,8	Relevant	0,95			
Item 5	19	0,9	Relevant	0,85			
Item 6	20	1	Relevant	0,9			
Item 7	18	0,8	Relevant	0,95			
Item 8	18	0,8	Relevant	0,95			
Item 9	19	0,9	Relevant	0,88	Pertinent	0,90	
Item 10	20	1	Relevant	1			
Item 11	18	0,8	Relevant	0,77			
Item 12	18	0,8	Relevant	0,85			
Item 13	19	0,9	Relevant	0,9			
Item 14	20	1	Relevant	0,95			
Item 15	17	0,7	Relevant	0,95			
Item 16	20	1	Relevant	0,88			
Item 17	19	0,9	Relevant	0,88			
Physical functioning (Karnofsky)							
Item 1	17	0,7	Relevant	0,95			
Item 2	20	1	Relevant	0,9			
Item 3	18	0,8	Relevant	0,8	Pertinent	0,91	
Item 4	19	0,9	Relevant	0,9			
Item 5	20	1	Relevant	1			

Legend: N*e* = the number of panel members indicating an item "essential"; CVR = Content Validity Ratio
I-CVIs = Content Validity Indices calculated at the item-level: S-CVI = Content Validity Indices calculated at each scale-level

score showed the 'usefulness' of a unique measure to frame the peculiarities of chronic patients.

Discussion

This study was mainly aimed to assess the content and face validity of a new proposal of comprehensive measurement of the level of available self-resources in patients with chronic diseases. This study provides solid basis for future explorations of the predictive characteristics of the D&CM score, as well as the assessment of its construct validity and reliability. Overall, D&CM score is functional in closing the current gap given by the unavailability of a comprehensive measure of determinants of wellbeing in chronic patients to determining an assessment of their dietary habits, physical resources, adherence, psychological status, ability to perform daily activities.

All the content validity scores showed the high level of relevance and pertinence of the selected pre-existing measures, thus no modifications were needed in relation to the proposed scales embodied into D&CM score. Accordingly, it could be argued that D&CM score has the potentiality to address the educational interventions to enhance the modifiable self-resources of chronic patients. The idea underpinning the development of D&CM score is consistent with the literature on educational interventions for chronic patients, such as the using of health coaching (30). Precisely, health coaching can be defined as an approach to helping patients gain their knowledge, skills, tools and confidence in becoming active and reaching their self-identified health goals (30). Consistently, the areas encompassed in D&CM score are those needed to plan a health coaching for patients with chronic diseases (31). In this regard, the high multi-dimensional nature of D&CM score could be useful to overcome the main limits of the tools used to assess the effectiveness of the coaching interventions (31, 32).

Further, D&CM score encompasses the main common areas of health determinants in chronic patients, being potentially useful for a wide range of chronic conditions, and overcoming some constraints of tools available only for specific clinical conditions, such as for chronic obstructive pulmonary disease (COPD) (33) or for patients with diabetes (34). Precisely, D&CM score is not intended to be a surrogate of specific scores (e.g. self-care scores), but it is developed to provide a comprehensive orientation for evaluating the general self-resources, especially in the setting of primary care (35).

Further tests of D&CM are needed to assess the cut-off to address the interventions. Particularly, it will be useful to choose an external validation parameter for describing the sensitivity and the specificity of D&CM score in relation to the selected external parameter (36). Further, it will be necessary to test the predictive propriety of D&CM score on some identified outcomes, such as re-hospitalizations. Overall, D&CM is promising, even if it is still under testing for more robust considerations.

Considering this study, the main limitation is related to the pilot nature of the aim. Thus, the results of this study have to be intended as the synthesis of the views of 20 experts on the content validity of D&CM score, with a poor possibility of inferential considerations. This limit is in line with the methodologies of content validity studies (25). However, this study has the worth to give solid basis for the further development of D&CM score, being a methodological description related to the score development and its initial validation process.

Conclusions

D&CM score shows evidence of content and face validity. It could be useful to assess the main common areas of self-resources in patients with chronic diseases, addressing educational intervention of health coaching. Future research has to provide more evidence of validity of D&CM score, describing its predictive performance in the different cohorts of chronic patients. Further, it is needed support with an external validation parameter for the study of D&CM score sensitivity and specificity. Clinicians working in the primary care settings might benefit of a comprehensive measure of assessment of patients' self-resources.

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Allergic rhinoconjunctivitis: pathophysiological mechanism and new therapeutic approach

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Summary. Allergic rhinoconjunctivitis (AR) is the most common IgE-mediated disease. A type2 immune response is involved in AR pathogenesis. Allergic inflammation is characterized by eosinophilic infiltrate and mediators release. AR treatment is usually based on medication prescription, including antihistamines and intranasal corticosteroids. However, medications may be prescribed for long periods and sometimes may be scarcely effective, thus aggressive strategy should be used. Therefore, complementary medicine is becoming attractive for patients at present. Nutraceuticals represent interesting therapeutic options in clinical practice. In this regard, a new compound has been designed containing Vitamin D3, *Perilla* extract, and quercetin. (www.actabiomedica.it)

Key words: allergic rhinoconjunctivitis, inflammation, immune response, nutraceuticals

Allergic rhinoconjunctivitis (AR) is the most common IgE-mediated disorder as its prevalence has exceeded the 40% of the general population; in particular, the most worrying aspect is that AR prevalence has approximately doubled over the past 20 years (1). Thus, the term Allergy Epidemic has been coined to emphasize this alarming phenomenon. Many hypotheses have been proposed to explain this exponential growing: the most updated is the Microbiota Hypothesis (2). This theory concerns the pivotal role exerted by the intestinal microbiota in manipulating the immune response, mainly in infancy and childhood. An altered composition of the human microbiota, namely a reduction of quality and quantity of microbial strains, impairs the physiological maturation of the immune system (3). In other words, the microbiota represents a reservoir of antigens that are crucial to stimulate the developing the immune function in infants and children (4). An adequate antigenic "pressure" is necessary to ensure the correct immunological maturation that is physiologically oriented toward a type1 polarization (Figure 1). Type1 immune response is necessary to guarantee a correct defence against infectious agents. Therefore, a defective composition, i.e. qualitative and/or quantitative, of the human microbiota promotes the maintenance of Type2-polarized immune response that is typical of the foetal period (5,6). Actually, the foetus would represent for the mother a non-self-antigen and consequently should be rejected. To avoid this negative situation, the foetal-placental unit develops a type2 milieu able to preserve the foetus from the potential maternal reject. The foetus grows therefore in an environment that is Type2-polarized and the infant maintains that arrangement. Therefore, the physiological maturation from a type2-polarized toward a type1-oriented immune response is promoted by a correct, such as quantitatively and qualitatively, antigenic exposure, insured by the "good" microbiota. However,

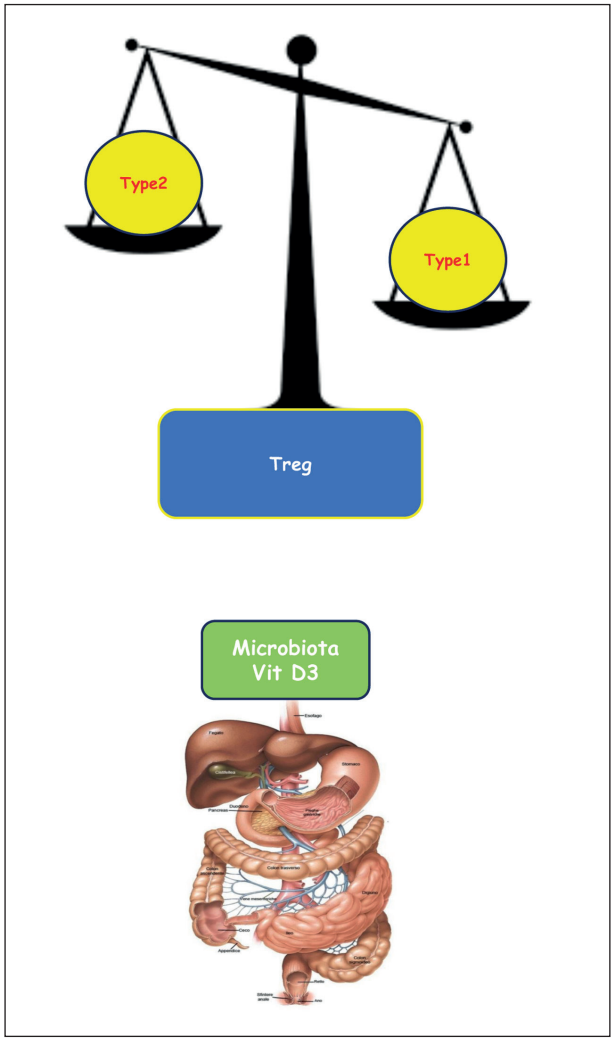

Figure 1. Balance between type1 and type2 immune response in healthy subjects. Treg= T regulatory cells

the present life-style, mainly occurring in the western countries, is characterized by a low fibre diet, vaccinations, reduced infections, antibiotics overuse, in other words a "over-hygienic" environment. This situation alters the normal composition of the microbiota, impairs the maturation of the immune system, and ultimately allows the increase of allergic disorders. In this context, Vitamin D_3 exerts synergic activity with microbiota to guarantee a correct immune function.

As explained, the immune response is dysregulated in allergic patients and is polarized toward the type2. The main cellular factor involved in this imbalance is the T regulatory cell subset. Allergic patients paradigmatically present an allergen-specific functional defect of T regulatory cell. This defect depends also on both impaired microbiota and Vitamin D_3 deficiency (2). However, this unbalanced arrangement may be reversible as may be corrected by specific treatments. The defect of T regulatory cells maintains the type2 polarization that results in the sensitization phenomenon, i.e. the ongoing production of immunoglobulin class E (IgE) characterized by the same allergen-specificity of T regulatory cells (2). The IgE are abundantly present on the surface of the primary effector cells, such as mast cell and basophil. When the allergen enters into the nose and the eye, it binds with the specific IgE, covering the mast cell surface, and immediately starts the allergic reaction (Figure 2). Really, this antigen-IgE link activates mast cell that releases pre-formed mediators, including primarily histamine, and cytokines, and produces neo-formed mediators, including arachidonic acid metabolites. Histamine is the main mediator involved in allergic reaction and is of primary importance

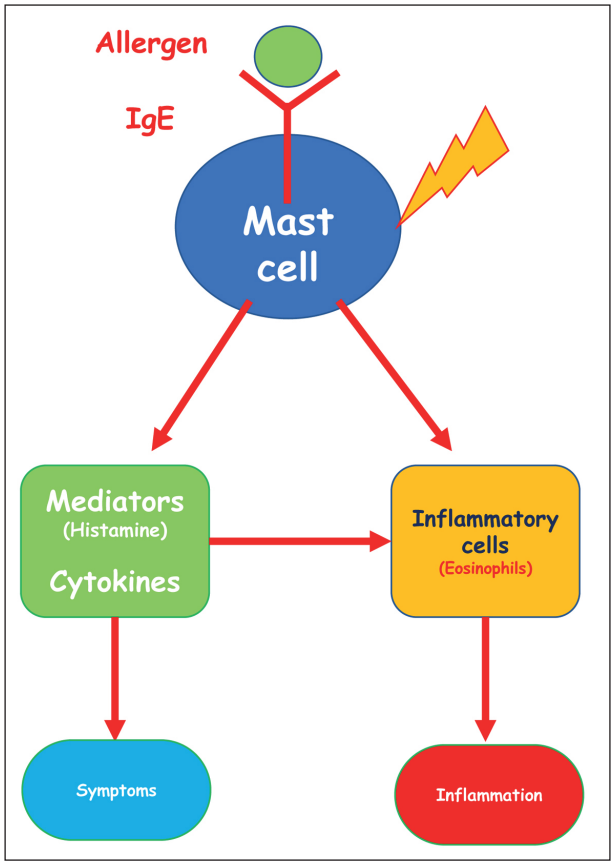

Figure 2. Allergic reaction consequent to allergen exposure

as it elicits all allergic symptoms. On the other hand, mast cell-derived cytokines induce the cascade of inflammatory phenomena typical of allergic inflammation. Allergic inflammation is in turn responsible to cause other clinical events, mainly concerning mucosal swelling and vascular congestion and leakage.

The pivotal pathophysiological characteristic of allergic reaction is the presence of a typical inflammatory pattern at the level of the target organ, i.e. the nose and the eye in AR patients (7). In particular, an abundant eosinophilic infiltrate represents the typical feature during allergic reaction. A fundamental concept has been evidenced some years ago: the persistence of allergic inflammation until the allergen exposure occurs. This paradigm is essential to recognize the need of using anti-inflammatory medication to treat allergic patients for long time.

From a clinical point of view, AR is characterized by nasal and ocular symptoms (such as ocular and nasal itching, nasal congestion, sneezing, watery rhinorrhoea, eye redness, and lacrimation), and also general complaints such as fatigue and cough. In particular, the most disturbing symptoms are nasal congestion and ocular itching.

AR has also detrimental effects on mood, sleep, social activities, work and scholastic performance. If there is asthma comorbidity, uncontrolled AR may aggravate the asthmatic symptoms. Moreover, quality of life is significantly impaired in children and adolescents with AR (7). Therefore, all allergic patients should be adequately treated.

In addition, AR is characterized by two clinically relevant aspects. First, AR frequently precedes the asthma onset as the airways share common pathogenic mechanisms (8). In addition, AR patients have more infections than non-allergic subjects because of an impaired type1 immune response and a mucosal inflammation that promote infections (9-11).

AR treatment is usually pharmacological, including antihistamines and intranasal corticosteroids, even though these drugs exert a merely symptomatic effect as do not cure allergy; in addition, medications could not completely relieve symptoms (12). However, it is well known that aggressive therapy and prolonged use of medications may produce significant side effects, thus these strategies should be limited or avoided altogether in children (13). As a consequence, more and more people prefer to use complementary medicine, for example herbal medications and vitamins (14). In this regard, there is a growing interest around nutraceuticals.

Nutraceuticals are just substances of natural origin that can have a positive effect on the state of health. At present, nutraceuticals, with proven efficacy, are popularly associated with conventional therapy to speed up recovery, make it long lasting, and avoid aggressive therapeutic regimens, including systemic corticosteroids, or at least limit their duration if they are needed (15,16). Their use is popular, but methodologically correct studies (randomized controlled trials, RCT) are very few.

Recently, a new compound has been developed for the treatment of allergic rhinoconjunctivitis: Lertal®. The current Supplement will present and discuss its components, the published evidence, and new experiences conducted in clinical practice.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Complementary treatment of allergic rhinoconjunctivitis: the role of the nutraceutical Lertal®

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Summary. Nutraceuticals represent interesting therapeutic options in clinical practice. In this regard, a new compound has been designed: Lertal®. It contains quercetin, perilla extract, and vitamin D_3. These agents exert anti-allergic and anti-inflammatory activities. This article reports and discusses the results of four clinical studies conducted in adult and paediatric patients suffering from AR. Outcomes provided evidence that Lertal® may significantly prevent clinical worsening when prescribed as add-on to continuous antihistaminic treatment and also prevent clinical exacerbations, such as the need of rescue medication, when used alone as preventive strategy in AR patients. (www.actabiomedica.it)

Key words: allergic rhinoconjunctivitis, nutraceuticals, quercetin, perilla, vitamin D3

A new compound has been recently developed for the treatment of allergic rhinoconjunctivitis: Lertal®. Lertal® is an oral food supplement, containing: *Perilla frutescens* 80 mg (as dry extract), Quercetin 150 mg, and Vitamin D_3 5 mcg (200 IU).

The dry extract of *Perilla frutescens* seeds contains rosmarinic acid and other flavonoids, such as luteolin, apigenin, and crhysoeriol; all of them have a well-documented *in vivo* and *in vitro* anti-allergic activity (1-5). Curiously, the leaves of *Perilla frutescens* are a popular garnish in Japan, as they are employed to antagonize fish and crab meat allergy, and also are used as a food colorant. Other medical indications include sedation and treatment of indigestion and food poisoning. About the anti-allergic activity of rosmarinic acid, a murine model demonstrated the significant suppression of passive cutaneous anaphylaxis reaction (5). A *Perilla*-derived methoxyflavanone also inhibited the *in vitro* IgE-mediated histamine release from a basophilic cell culture and *in vivo* prevented allergic rhinitis-like nasal symptoms in a murine model of Japanese cedar pollinosis (6). *Perilla* also has significant inhibitory activity against both 5-lipoxygenase and 12-lipoxygenase, key enzymes in one of the pathways of allergy and inflammation (7). Luteolin has been recognized as an antioxidant scavenger of damaging free radicals and inhibits protein kinase C, i.e. key regulator of inflammatory events and smooth muscle constriction (8). Luteolin is also a potent inhibitor of mast cell activation as could completely block the release of histamine and pro-inflammatory cytokines (9). Luteolin also reduced IL-4 and IL-5 and increased IFN-γ at bronchial level in an asthma model (10). Apigenin is another flavonoid able to suppress IgE and IL-4 production (11).

Quercetin is a bioflavonoid found in red wine, grapefruit, onions, apples, black tea, and, in lesser amounts, in leafy green vegetables and beans (12). Quercetin has a strong affinity for mast cells and basophils and tends to stabilize their cell membranes, so blocking degranulation, and inhibiting the release of pro-inflammatory mediators and cytokines implicated in allergic inflammation (13,14). In particular, a placebo-controlled study showed that 8-week querce-

tin course significantly reduced ocular symptoms in patients with Japanese cedar allergy (15). Using the same clinical model, a preventative activity was also documented on conjunctival symptoms (16). Quercetin significantly affected the nasal production of nitric oxide (17). Moreover, quercetin inhibited the *in vitro* activation of eosinophils (18). Therefore, all these outcomes confirm and underline its anti-allergic activity.

Vitamin D_3 is important for its contribution to the normal function of the immune system (19,20). In particular, it has been evidenced a relevant role in both prevention and potential treatment of AR, as it restores physiological T regulatory activity and exerts also anti-inflammatory activity as widely reported (21-24). In addition, it has been reported that Vitamin D_3 serum level is inversely correlated with immunological biomarkers of inflammation, such as IL-6 and IL-10 (25). Another intriguing anti-allergic mechanism of Vitamin D_3 has been demonstrated in mast cells: actually, mast cell can actively metabolize Vitamin D_3 to self-modulate IgE-mediated activation (26).

In addition, there is another interesting technological characteristic: Lertal® is formulated in bilayer tablets composed of a fast-release layer that allows the rapid antihistamine activity of *Perilla*, and a slow-release layer that enhances Quercetin and Vitamin D_3 bioavailability and anti-allergy activity spread over time. Thus, Lertal® could be considered as a fast-slow release compound.

The role of Lertal® in the treatment of Allergic Rhinoconjunctivitis

An important premise should be considered: nutraceuticals cannot replace completely standard pharmacological treatment to quickly relieve symptoms, but could be used to improve standard treatment or to prevent possible clinical relapse. Indeed, it is well known that a possible symptom worsening may occur also during the active antihistaminic treatment or after its suspension, as expression of insensitivity, tachyphylaxis, or excessive allergen exposure. Therefore, nutraceutical could be used as add-on strategy or preventative treatment.

At present, there are 4 published articles concerning the use of Lertal® in patients with AR: two studies concern adult patients and two trials enrolled allergic children.

Adult studies

A recent open study, conducted in adult patients with seasonal AR, showed that Lertal® treatment induced a significant reduction of both symptom severity and consumption of anti-allergic drugs (27).

This clinical study was performed to demonstrate the efficacy of Lertal® for the relief of nasal and ocular symptoms and the reduction of anti-allergic medications use in patients with AR.

Twenty-three patients (16 women, mean age 44 years, and 7 men, mean age 46 years) were enrolled in this trial. Patients had history of AR symptoms for at least 1 year and were sensitized to *Parietaria officinalis* pollen. At baseline, patients were symptomatic. A total symptom score (TSS) was used to score the daily symptoms' episodes by a four-point scale (0=no episode; 1=1-5; 2=6-10; 3=≥11 episodes/day). Patients were visited at baseline, such as before the treatment, and after 1 month of the nutraceutical supplementation. Symptoms were assessed at both visits; the use of anti-allergic medications was also recorded. Lertal® was given to the patients together with indications of its use: to be taken twice a day, morning and evening, during or after meal, for 30 consecutive days. Pollen count was also carried out to document the related clinical feature during the study period.

There was a reduction of approximately 70% for symptom scores and 73% for the anti-allergic use. In particular, there was a significant reduction of both TSS ($p<0.001$) and single symptoms ($p<0.0001$ for all symptoms, i.e. sneezing, rhinorrhoea, nasal obstruction, ocular itching, lacrimation, and conjunctival congestion). Notably, there were no noteworthy adverse events during the study.

Another study was conducted in patients suffering from seasonal allergic conjunctivitis (SAC) using an ophthalmological formulation, such as Ophthalmic Lertal® spray (28). This medical device contains *Perilla frutescens* extract, hyaluronic acid, and liposomes. Hyaluronic acid is a naturally occurring linear disaccharide

polymer with lubricating and rehydrating properties commonly used in the management of dry eye syndrome (29). Liposomal eye sprays may provide symptomatic relief for SAC, which often causes a tear film deficiency, by stabilizing the tear film lipid layer (30).

Therefore, this open-label clinical study aimed at investigating the efficacy and safety of ophthalmic Lertal® spray in patients with SAC. Concomitant use of anti-allergic medications, including topical or oral antihistamines or corticosteroids or topical decongestants, was permitted.

This was a 4-week, open-label, single-arm, uncontrolled trial. Patients (17 females and 13 males, mean age 43.4 years) were consecutively enrolled during the peak pollen season. Patients applied Lertal® spray to the closed eyelid three times daily (morning, midday and evening); additional doses were applied as needed for acute SAC signs and symptoms.

Patients underwent two clinical visits; a baseline visit (Visit 1) and an end of study visit (Visit 2; i.e. 4 weeks after starting study treatment). Ocular signs and symptoms were recorded using the Total Ocular Symptom Score (TOSS) scale (where 0 = no symptoms; 1 = mild symptoms; 2 = moderate symptoms and 3 = severe symptoms) for the following nasal and ocular signs and symptoms: ocular itching, lacrimation, conjunctival congestion, ocular hyperemia and photophobia. Symptoms were assessed by clinicians at Visits 1 and 2.

The primary efficacy endpoint was the change in mean TOSS (i.e. ocular signs/symptoms) from baseline after 4 weeks of study treatment.

Secondary efficacy endpoints included: change from baseline in individual ocular symptom scores; change from baseline in the daily use of anti-allergy medications; and patient assessment of how 'pleasant' the spray application felt.

For the assessment of the changes in concomitant use of anti-allergy medications, patients were divided into two groups: the first group continued taking anti-allergy medications as needed, and the second (with lower baseline usage due to less severe and disabling symptoms) were instructed to discontinue anti-allergy medications (rescue treatment was permitted).

After 4 weeks of Lertal® spray administration, there was a significant reduction in all ocular signs and symptoms from baseline (mean ± _SD TOSS 10.0 ± _3.24 at visit 1 vs. 3.7 ± _2.25 at visit 2; P<0.001) among patients with SAC, corresponding to a 63% reduction in mean total symptom score.

Mean scores for the individual ocular symptoms were all significantly reduced from baseline at the end of treatment, with reductions from baseline in mean symptom scores of 56% for ocular itching, 58% for lacrimation, 63% for ocular hyperemia, 66% for conjunctival congestion and 85% for photophobia. With regard to changes in TOSS scores for individual patients, 27 patients (90%) showed improvements from baseline at the end of the treatment, while three patients (10%) were considered non-responders.

Among patients who continued concomitant use of anti-allergy medications (n=15) due to the severity of their symptoms at the beginning of the study, there was a significant (P<0.001) reduction in the mean overall daily use of all medications and of each individual drug class with percentage reductions in mean daily usage scores of 64% to 100%. In a subjective assessment of how pleasant the spray application felt, 55% of patients answered 'very pleasant', 30% answered 'pleasant' and 15% answered 'acceptable'. In particular, the majority of patients described a pleasant feeling of coolness in the ocular area and resolution of the itching a few minutes after the application of the spray.

No adverse events were observed during the 2-hour period following Lertal® spray administration. Overall, during 4 weeks' treatment, no clinically relevant adverse effects were reported.

Paediatric studies

It is well known that AR is common mainly in childhood and in adolescents. The medical treatment is substantially the same used in adults. However, particular attention should be paid about medication overdosing and adverse events. In this regard, nutraceuticals could play an interesting role as complementary therapy in order to save medication use and so minimize adverse events. Therefore, two hypotheses should be tested to validate the usefulness of this nutraceutical in AR treatment: i) to investigate its capa-

bility to amplify the response to standard antihistaminic AR medications and/or reduce the insensitivity during the active treatment, and ii) to demonstrate its potentiality to prevent possible relapse after standard treatment suspension. To answer to these unmet needs, a polycentric, randomized, Italian study has been performed in two phases: the first as double blind, placebo-controlled trial during standard active AR treatment and the second as an open-label, parallel-group, extension study after the standard treatment withdraw.

First phase study

Thus, the aim of the first phase was to evaluate the efficacy and safety of Lertal® as an add-on treatment in children with AR (31). The first phase was a 4-week, randomized, polycentric, double-blinded, parallel-group, placebo-controlled trial. One hundred and sixty patients suffering from AR were planned for enrolment in 17 Italian Paediatric Allergy clinics. AR diagnosis was performed, according to validated criteria (32), such as if nasal symptom history was consistent with documented sensitization.

Inclusion criteria were: age range 6-12 years, AR diagnosis, sensitization to house dust mites or pollens, Total Symptoms Score (TSS) ≥ 15 and at least 1 for nasal congestion, written informed consent of patients and of parents or legal guardians. TSS is the conventional way to measure symptom severity as it is used in all methodologically correct trials.

Exclusion criteria were: uncontrolled asthma, secondary rhinitis to other causes, concomitant acute or chronic rhinosinusitis, nasal polyps, current use of topical or systemic corticosteroids, antihistamines, antileukotrienes, inadequate washout of them, nasal anatomic defect, respiratory infections in the last 2 weeks, participation in other clinical studies in the last month, documented hypersensitivity to the study product or its excipients, and trip planned outside of the study area.

After 2-week run-in period, eligible patients were randomly (1:1 ratio) treated with Lertal® (1 tab/day for 4 weeks) plus standard antihistamine therapy or Lertal® placebo (1 tab/day for 4 weeks) plus standard antihistamine therapy (Figure 1). Systemic or intranasal corticosteroids, leukotriene antagonists, and sodium cromoglicate were prohibited during the study. Four visits were performed: Visit 1 at run-in, Visit 2 at baseline (W0), Visit 3 after 2 weeks (W2), and Visit 4 after 4 weeks, i.e. end of treatment (W4). The study protocol was approved by the Ethics Committees of each center. The study was registered at ClinicalTrials.gov ID NCT03365648.

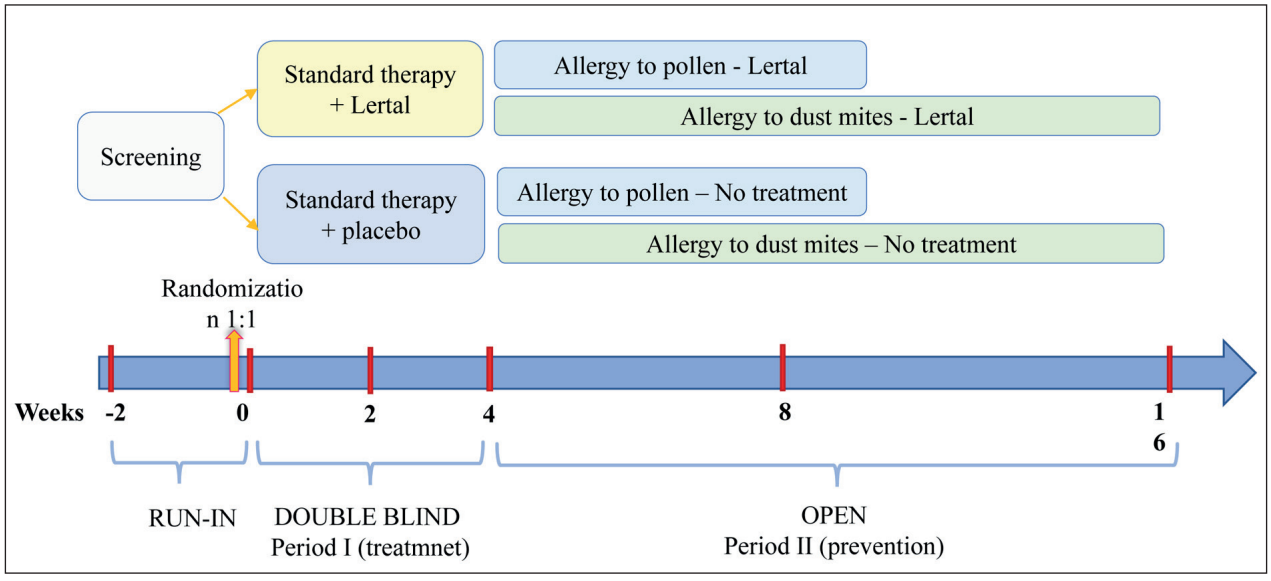

Figure 1. Study design of the pediatric trial

The primary endpoint of this study was the TSS change from the baseline to the end of the treatment (4 weeks). The secondary objectives included: overall symptom control assessed by means of a VAS after 2 and 4 weeks of treatment, change from baseline of the Total Symptom Score (TSS) after 2 weeks of treatment, number of responders (at least 30% reduction of TSS) after 2 and 4 weeks of treatment, time to maximum effect on TSS vs placebo, change of TSS from 2 and 4 weeks (worsening was defined as at least 30% increase of TSS), use of rescue treatment, change from baseline of Total Nasal Symptom Score (TNSS), Total Ocular Symptom Score (TOSS) and Total Throat Symptom Score (TTSS) after 2 and 4 weeks of treatment, issues interfering with quality of life at baseline and after 4 weeks, duration of symptom-free or with mild symptoms.

Nasal symptoms (TNSS) included itching, sneezing, rhinorrhea, nasal congestion; ocular symptoms (TOSS): itching, hyperemia of conjunctiva, tearing; throat symptoms (TTSS): itching, coughing. With the help of their parents, patients scored symptoms severity on a 4-point scale: 0 = absent or irrelevant, 1 = mild, 2 = moderate, 3 = severe.

At Visit 3 and Visit 4 the patient was asked to indicate overall system distress on a 100 mm Visual Analogue Scale (VAS) were 0 is equal to no discomfort and 100 the worst possible discomfort.

The Pediatric Rhinoconjunctivitis Quality of Life Questionnaire (PRQLQ) consists of 23 questions in 5 domains (nasal symptoms, ocular symptoms, practical issues, limitation of activities, other symptoms), that are answered on a 7-point scale (0-6), where 0 represents the absence of problems and 6 the greatest symptom distress. Children completed the questionnaire together with a parent at baseline and at Visit 4.

Safety was assessed on the incidence of adverse events for each treatment and on physical examinations.

The TSS at baseline was 15.9 (± 1.7) in Lertal®-group and 16.1 (± 1.2) in Placebo-group (p= n.s.). Both groups significantly (p<0.0001 for both) reduced TSS (last 12 hours) after 2 and 4 weeks, without between-group difference. In particular, TSS was at W4: 5.83 (± 4.5) in Lertal®-group and 6.39 (± 4.38) in Placebo-group (p=n.s.). There was a trend between groups about the percentage variation change: - 63.6% in Lertal®-group and – 60.7% in Placebo-group.

Notably, 24 children had total symptom score worsened (i.e. ≥30% increased TSS) between W2 and W4: 8 in Lertal®-group and 16 in Placebo-group, being the difference between treatments significant (P<0.05), as reported in Figure 2. In particular, the proportion of patients with maximum effect on TSS (i.e. the last 12 hours) at W2 was higher in the Placebo-group respect to the Lertal®-group (50.77% and 39.06% respectively). The proportion of patients with maximum effect on TSS (last 12 hours) at W4 was higher in the Lertal®-group respect to the Placebo-group (60.94% and 49.23% respectively). This trend in the differences of the proportions (time to maximum effect) is not due to a faster effect of the Placebo-group but to the fact that the proportion of worsened patients (i.e. >=30% increase of TSS between W2 and W4) was significantly higher from Week 2 to Week 4 in the Placebo-group than in the Lertal®-group.

Both treatments were well tolerated and no serious adverse events were reported.

It is well known that AR treatment is addressed to symptom relief and inflammation control. Antihistamines are the first-choice treatment in childhood, but, if they are ineffective, corticosteroids represent the second-level option, nevertheless many parents exhibit "steroid-phobia". As pharmacological medications are only symptomatic, and potentially may cause adverse

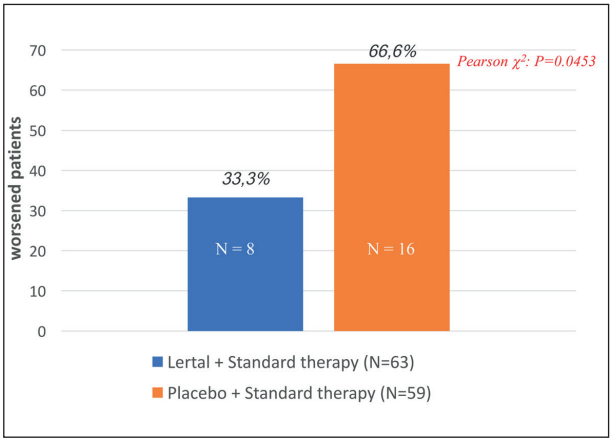

Figure 2. Outcomes of the Phase I: number (and percentages) of patients with clinical worsening (TSS increase ≥ 30%) between W2 and W4 in active and placebo group

events, there is growing interest by both doctors and parents about complementary therapeutic strategies. This study confirmed that standard antihistaminic treatment improved the clinical feature in children with AR. Furthermore, it has been evidenced that Lertal®, used as add-on therapy, was able to tendentially improve the effect of the standard AR treatment. Actually, the Lertal®-group achieved a mean reduction of about 64% of symptom severity, whereas the Placebo-group 60%. However, it has to be noted that a relevant difference was not expected because of both the obviously antihistaminic activity and the intrinsic characteristics of the nutraceutical. Notably, Lertal® significantly reduced the possible occurrence of intercurrent clinical relapse during the standard treatment in children with AR. Indeed, the most important finding of this study was the capability of Lertal® to prevent symptom worsening during conventional antihistaminic treatment, mainly during the second period of the treatment (from week 2 to week 4). It is well known that some AR patients are partially responder, resistant, or develop tachyphylaxis to medications. In this regard, it is clinically relevant to identify the pathogenic mechanisms involved in these patients. In the current trial, 24 patients (8 in Lertal® group and 16 in Placebo group) had a clinical relapse between the third and the fourth week, despite an initial clinical improvement. Therefore, the possible explanation of this behaviour could depend on the common characteristic of these children, such as all of them had poly-allergy. It means that patients with allergy to more allergens, i.e. pollens and perennial allergens, usually present more severe symptoms than mono-allergic patients (33). Consequently, these poly-allergic children had clinical worsening, despite ongoing treatment, as exposed to multiple allergens and so developing more severe allergic reaction. Nevertheless, this study shows that the add-on Lertal® preserved clinical relapse in a larger number of poly-allergic children than standard therapy. This finding is particularly interesting if contextualized as part of a continuous effective antihistaminic treatment and this preventive activity may allow to avoid the recourse to corticosteroids.

This outcome could be explained by the multifaceted mechanisms of action exerted by Lertal®. In particular, Lertal® effects seem to be grounded in the complex anti-inflammatory and anti-allergic activity exerted on the immune response by the three compounds.

Another remarkable point was the interest, in other words the sensitivity, to perform a rigorous study before in childhood than in adulthood. This point deserves attention as there is relevant lack of paediatric studies that evaluate the efficacy and the safety of treatments in the paediatric age.

Therefore, the present study documented that add-on Lertal® treatment was able to: i) partially improve standard AR treatment in children, ii) significantly prevent the occurrence of clinical worsening in a subgroup of poly-allergic children, and iii) be safe.

Second phase study

The second phase was designed as open and parallel-group study and was conducted after the end of the blind-period (34). It was a 4-12-week open-label, parallel-group, extension study in which patients treated with study product in Period I continued treatment with Lertal® tablets, whereas patients initially treated with placebo received no further treatment.

Continuous treatment with systemic or intranasal antihistamines, corticosteroids, leukotriene antagonists and sodium cromoglicate were prohibited during the study. Two visits were scheduled during this period to collect efficacy, safety and quality of life data. Patients were asked to return their diaries at these visits in order to collect data concerning exacerbations and or adverse events.

The end-points of the Phase II were the length of time symptom-free or with mild symptoms, and the number, intensity, and duration of exacerbations. Exacerbation was defined as the need of restarting an antihistamine medication of any kind, at any dose and of any duration.

Safety was assessed by the incidence of adverse events for each treatment and by physical examinations.

The Phase II study included a total of 128 patients, of which 64 assigned to open Lertal® therapy (Lertal® Group: LG) and 64 to observation alone (Observation Group: OG).

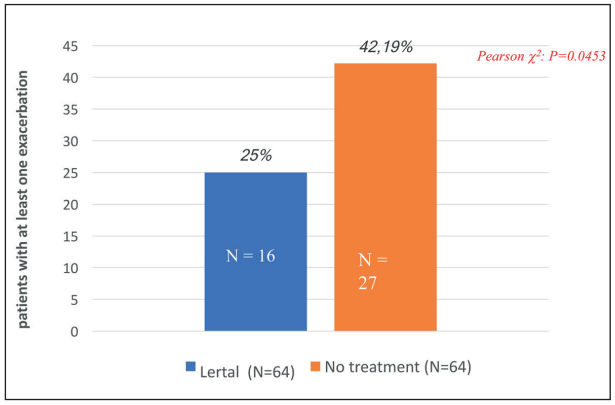

Figure 3. Outcomes of the Phase II: number (and percentages) of patients with at least one clinical exacerbation (need of antihistamines) in Lertal and control group

The two groups were homogeneous as far as age, gender, BMI, type of allergy, time from diagnosis and symptom severity are concerned at baseline.

The LG showed a significant difference concerning the duration of symptom-free days in comparison with OG (Log-Rank test = 4.16; p=0.0413) with a HR 0.54 (CI 95% 0.29-0.99).

Considering the number of children who experience an AR exacerbation, there was a significant difference between groups as only 16 children (25%) in the LG had an AR exacerbation, whereas 27 children (42.2%) of OG had an AR exacerbation (p=0.039), as shown in Figure 3. Analysing only the children with AR exacerbation, the total number of days in which each patient took at least one rescue medication was

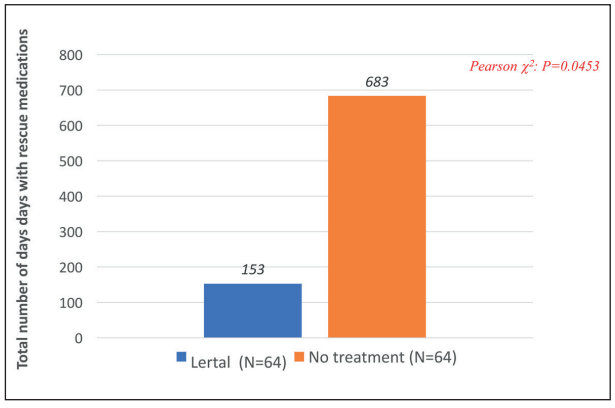

Figure 4. Outcomes of the Phase II: total number of days with rescue medication in Lertal and control group

significantly (p=0.018) lesser in LG than OG (9.6 ± 9 days and 28.5 ± 27.2 days respectively). Considering the global population, the cumulative days treated with rescue medication was significantly (p<0.0001) higher in OG than in LG (683 days and 153 days respectively).

Analysing only children treated with concomitant medications, LG children had tendentially less AR exacerbations than OG children (7 vs 12; p=0.051) and consequently used less antihistamines, such as had less days with antihistamines (Figure 4).

Lertal® treatment was well tolerated and no clinically relevant adverse events were reported.

Discussion of the outcomes

Noteworthy, it is important to consider that the clinical effect of a single dose of an antihistamine usually lasts until 24-36 hours, then symptoms reappear promptly (35). Similarly, the duration of intranasal corticosteroids effects is very short-lived after suspension, such as in a few days symptoms and inflammatory events recur (36). Moreover, both antihistamines and intranasal corticosteroids may be unable to completely inhibit allergic reaction in some circumstances, such as highly allergic patients, intense allergen exposure, or interfering disorders. Therefore, the use of add-on medications could be fruitful in such situations. Actually, the favourable effect, exerted by Lertal® in the first phase, was also evident in the second part of the active treatment, such as between the third and the fourth week, when some patients, after an initial response to drug treatment, showed a symptom worsening.

The outcomes of the Phase II not only confirmed indeed the favourable effects observed in the first phase, but also highlight a more relevant preventive activity consequent to the prolonged use of Lertal®. In particular, we would underline two main issues. The highly favourable HR value of 0.54: it means that the risk of AR exacerbation had been reduced in children taking Lertal® for a 4-12-week period by 46% in comparison with children without preventive intervention after the suspension of the standard 4-week antihistamine treatment. This finding is consistent with previous studies conducted in patients with asthma and in

children with allergic rhinitis (37-39). Therefore, the second part of present study evidenced that Lertal® treatment was able to approximately halve the risk of AR exacerbation after one-month of antihistamine treatment. This outcome is also supported by the larger number of Lertal®-treated children (75%) who did not experience AR exacerbation than untreated children (58%). Notably, the total number of days with rescue medication, such as use of antihistamines, was significantly higher in untreated children. Consistently, the severity of symptoms was lower in Lertal®-treated children

In addition, these findings are consistent with outcomes documented in the first phase, such as Lertal®, used as add-on therapy, was able to tendentially improve the effect of the standard AR treatment and especially Lertal® significantly reduced the possible occurrence of intercurrent relapse during the standard treatment in children with AR. In this regard, it is noteworthy to consider that some children did not continue to be well controlled by antihistamines despite the fact that antihistamines have also an antiallergic activity (40-45). Therefore, the current results could be envisaged as a proof of concept that Lertal® provides its preventive activity by an additional antiallergic activity.

Another important aspect concerns the adverse events. In this regard, no patients experienced serious treatment-emergent adverse events or fatal adverse events. Only 2 children of the active group reported suspected treatment-related adverse events with temporarily discontinuation in the Phase I and only 1 of the Lertal® group in the Phase II. Anyway, all adverse events were mild and self-resolving.

The strength of this study was the methodological accuracy, based on the double-blinded, randomized, parallel-group, and placebo-controlled design of the first phase, the presence of a successive observational period, the sample size estimate.

From a clinical point of view, Lertal® could be considered a preventive compound that could be favourably prescribed both as add-on therapy during continuous antihistaminic therapy and as preventive strategy alone. As the safety profile is optimal, the duration of Lertal® treatment could be continued for prolonged periods as long as the pollination season in pollen-allergic patients or fall-winter in mite-allergic subjects.

In prospect, other potential aspects could be considered: the impact on asthma co-morbidity and the prevention of respiratory infections. Asthma is frequently associated with AR and it may be favourably improved by anti-allergic treatments that control respiratory inflammation (46,47). Moreover, allergic patients, as previously reported, may frequently contract infections that may be reduced by antiallergic treatments (48).

Another relevant issue should be considered: to document efficacy and safety of any AR treatment, evidence based medicine needs randomized controlled trials. So rigorous methodology has to be applied to the protocols, including patient's characteristics, inclusion and exclusion criteria. In this regard, the age is frequently a sensitive parameter. However, AR pathogenic mechanism, clinical features, and responsiveness to medications are shared by children and adults in a specular manner. Actually, many trials, including pivotal clinical trials, have been conducted enrolling both children and adults. The findings were not conflicting after stratifying for age. Therefore, there is reliable consistency between paediatric and adult outcomes in randomized controlled trials.

On the basis of this background, it is conceivable that outcomes obtained by trials in children with allergic rhinitis can be extrapolated and applied in adults with allergic rhinitis.

Therefore, there is convincing rational and proof that the results provided by the Lertal® studies may be conveniently extended to adult patients suffering from allergic rhinitis.

Conclusions

Nutraceuticals will play a relevant role in the future treatment of AR, but their use cannot be separated from the proved evidence of their effectiveness and safety. In this context Lertal® meets these requirements. In particular, there is evidence that Lertal® may be favourably used to prevent clinical worsening as add-on strategy and to reduce clinical exacerbations as mere preventative strategy.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Cosmotellurism in Lombroso's work

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Summary. *Background and aim of the work:* Few know that Lombroso was also involved in epidemiological research. In particular, Lombroso's scientific reflections on Medical Geography were addressed to the theme of climate influences and meteorological conditions on human conduct. The authors analyze the scientific production and the works of Lombroso devoted to medical geography. *Discussion:* Lombroso carried out accurate epidemiological investigations using the statistical method with great modernity, combining health data with geographical and climatic data to demonstrate the relationship between man, the environment and health in a social vision of preventive and curative medicine. *Conclusions:* The theory of Cosmotellurism in Lombroso's work is not only a source of unquestionable interest in the History of Medicine. The heritage of Medical Geography within the pre-bacteriological medical culture can continue with its teachings to correctly address the clinician's thinking even in the current historical context in which endemic and epidemic pathologies re-emerge in various parts of the world. (www.actabiomedica.it)

Key words: Lombroso, Medical Geography, Cosmotellurism, nineteenth-century's epidemiological research

1. Introduction

Within the variegated and ephemeral theories proposed by medical doctrine in the nineteenth century, the theory of Cosmotellurism is of relevance to the work of Cesare Lombroso.

This term (composed of cosmos and telluric) refers to factors relating to the physical environment such as, for example, climate, seasons, temperature, soil structure, agricultural production, and weather conditions.

In his extensive scientific production, the works devoted to medical geography are perhaps the least known.

The psychiatrist and criminal anthropologist, who became famous for his thesis of atavism (1, 2), totally repudiated and abandoned by the scientific community, had already taken a keen interest in this field of study at the beginning of his scientific career. In his volume *Studi per una geografia medica d'Italia*, (*Studies for a medical geography of Italy*) published in Milan in 1865, Lombroso proposed a conception of health that was firmly grounded in data from regional nosographies (often with the aid of the historical method) and which took into account geology, atmospheric science, phytology and zoology (3, 4).

Lombroso's thought on the theme of the influences of climate and meteorological conditions on behaviour developed constantly throughout his scientific activity and is fully expressed in the volume *Pensiero e meteore. Studi di un alienista* (5, 6).

It should also be noted that, in the first two editions of the *Criminal man*, atavistic regression was not regarded as the only cause of crime (7-9). Indeed, Lombroso emphasized that this feature interacted with a number of other factors (such as occupation, age, education, nutrition and vices) including the urban environment and the climate (10), which favoured the re-emergence of primordial traits (11). Next to the

"internal" causes, innate or acquired (chronic alcoholism, head injury, diseases affecting the cerebrospinal axis), Lombroso identified "external" causes as climatic influences, along with dietary and social ones.

Of the climatic causes, the most important was considered a high temperature, while among social and dietetic causes, the most important was identified in alcoholic beverages. The preponderance of external causes determined a less serious delinquency and a possible correction, while that of internal causes generally determined a more serious crime and not susceptible to cure. In his decades-long research activity, Lombroso carried out accurate epidemiological investigations using a statistical method, combining health data with geographical and climatic data, demonstrating the relationship between man, environment and health, in a social vision of preventive and curative medicine (3-5). During this research, his attention focused on certain diseases such as cretinism, goitre and pellagra, demonstrating how a poor diet could favour them and make them proliferate (12).

On the basis of the importance attached to medical geography, he attempted to make statistical analyzes on the frequency of genius (that Lombroso believed was a condiction closely connected to madness, as such as a manifestation of a same underlying organic regression), on the diffusion of certain diseases and on the propensity to criminal behaviors. He then correlated these with environmental and climatic conditions, in order to explain the clinical and pathological data (13).

Lombroso's recognition of the action of geomorphological characteristics in the development of factors harmful to man was also reflected in his full adherence to the teachings of antiquity, as emerges from observations on the thought expressed by Hippocrates.

Hippocrates writes:

You will find that, in general, the appearance and manners of men conform with the nature of the territory. Where the land is fat, soft, rich in water, with very shallow waters (so as to be hot in summer and cold in winter), with a good climate, men will also normally be fleshy, without joints, humid, little inclined to fatigue and faint-hearted: they are prone to indolence and drowsiness; their limbs are obtuse, not thin and acute. Where the territory is bare, open, harsh, scourged by the winter and burned by the sun, you will find hard, dry, well-jointed inhabitants, tense and hirsute; in their nature you will find capacity to act; as to character and temperament, they will be proud and independent in judging, closer to wildness than to meekness; with regard to the arts, they will be more acute and intelligent, better in war. And you will find that everything that lives in that territory will be similar to the territory (14).

Lombroso writes:

A marvelous passage that summarizes in a few words all the studies of Quételet, of Montesquieu, of Cabanis (5).

According to Lombroso, the influence of pathological factors on human health was therefore closely connected with the geography of the area, particularly the topographical conformation of the territory (mountainous, flat, hilly, desert), and with climatic factors (15).

Certainly, the foundation of this conception is based on a historically consolidated thought in medicine, i.e. that the environment consists of a set of interactions between physical-chemical conditions and living beings, interactions that characterize a specific area (16).

2. Towards an ecosystemic thought

This conceptual choice remained substantially intact for several centuries before acquiring a precise scientific foundation in the experimentalism of the nineteenth century, when positivism dominated in both science and in philosophy.

The term cosmotellurism has been almost completely lost today; in nineteenth-century medical geography, however, it enjoyed an almost unconditional consensus (16).

One of Lombroso's undeniably original features is that, unlike the authors who had preceded him, he identified different "zones" in Italy; these he called "northern", "southern", "coastal" and "cosmotelluric" (divided into valley, volcanic, alpine and miasmatic areas). All these "zones" were characterized by the prevalence of different specific pathologies.

Lombroso also believed that "urban" areas should be regarded as autonomous and different from other areas, not only because they were the subject of more numerous and accurate studies, but also because of the presence of diseases that were less frequent elsewhere.

The conviction that the characteristics of the land influenced human physiology prompted the study of the composition of the land on which the various communities lived, since these characteristics were held to influence human and moral morphology.

Adherence to this concept led physicians to devote meticulous attention to mineralogy and chemistry and to study the composition and properties of soil and rocks and the transformations determined by the action of many natural and artificial factors. The importance attributed to the relationship between the quality of the land and certain diseases (17-20), particularly gastrointestinal diseases, was also highlighted by medical hygienists (21, 22).

Figure 1. Climatological Map of Italy from the text of Giacomo Barzellotti, 1838. "Avvisi" agli stranieri che amano di viaggiare in Italia o dimorarvi per conservare o recuperare la salute. Vincenzo Batelli e Figli, "Firenze"

The conviction that climatic factors influenced human health had been part of medical doctrine throughout the centuries, and was further consolidated by the observations of commercial or colonial travellers and explorers in Africa, Asia and the Americas.

Reports from these journeys show that Europeans who were not accustomed to these areas fell sick in "hot lands", while in "temperate lands" they did not run any health risks, except in the vicinity of stagnant water (23, 24). The so-called "cold lands", high above sea level, did not pose serious risks for the visitor. Obviously, knowledge of infectious diseases and their etiology was still in an embryonic phase.

Lombroso argued that there was a need for statistical research, in order to draw a nosological map of Italy or, at least, to sketch its main lines. In particular, he underlined the persistent differences in diseases and their diffusion in the various regions of the country.

He showed that special areas, so-called, "unhealthy" could be identified in the various regions and that these were influenced by local factors. This was particularly true for the "cosmotelluric areas", which were divided into valleys, volcanic areas, alpine areas and miasmatic areas.

As part of this research, and of considerable importance, especially for their socio-economic reflexes and the debates that followed, his studies on the endemic presence in various Italian provinces of cretinism, goitre and pellagra. Of the first two morbid forms, making use of observations begun in 1858 in Verona and continued with the examination of the subjects called to military service in Genoa, he identified the etiological relationship between the thyroid dysfunction, which was mainly dependent on water quality and the environmental and family factors.

He described the clinical characteristics of the diseases and indicated prophylactic measures, based on assumptions that would have proved to be scientifically unfounded.

As publicly stated by one of Lombroso'pupil, this toxic-zeist interpretation of pellagra has, unfortunately, delayed the adoption of effective measures by the authorities, prolonging the pellagra epidemic in Italy and causing the appearance of many new cases, hospitalizations in asylum and deaths (25).

His conclusions were reported in the entry "Cretinism" written for the Italian medical encyclopedia, including also elements of his by now begun studies of experimental anthropology (5).

With regard to the cosmotelluric areas, Lombroso observed a series of very specific infirmities in the valleys of the great Italian mountain chains, where "ozone, positive electricity" and light were scarce. The water that flowed down from the glaciers and gushed from the limestone rocks was often "stripped of carbonic acid and iodine", but full of calcareous salts. In those valleys, rickets, goitre and cretinism abounded (16).

Very precise data on cretinism were available, and these were compared with those on goitre. What emerged was a singular parallelism between the two infirmities, with a notable difference in the diffusion of these disorders among the various regions.

In the northern part of Italy, Piedmont and Lombardy were the most affected regions; in the province of Florence, by contrast, these disorders were almost completely absent. What particularly emerged from the analysis of the data was the almost complete absence of sufferers in the islands, except for some localities in the interior and in mountain gorges. The most affected areas were the mountain villages of the Alps and Apennines, where a large number of goitres were recorded (26).

The geophysical characteristics that Lombroso considered particularly significant for water were permeability, heat absorbency, greater and lesser ease of drying, "toughness" and porosity.

With regard to the composition of the soil, it seemed important to distinguish the various types: granitic, clay, sandy, muddy, loamy and volcanic soil. Swamplands and some stretches of water were considered to have harmful effects. Clay mixed with drinkable water seemed to be detrimental to the health, as doctors had observed wherever people lived on clay soil, and not only in swampy areas.

In the mid-nineteenth century, issues concerning cosmotellurism were the subject of wide debate in life sciences.

In the opinion of physicians, malaria provided an example of the cosmotellurism theories summarized in medical geography. Indeed, its onset was closely connected with an extraordinary concomitance of factors related to air, temperature and soil composition.

Regarding "mal'aria", the places where the disease occurred most frequently were investigated, as were their relationships with intermittent fever and the other manifestations of the disease.

After identifying the particular "unhealthy" areas of the various regions according to the presence of certain common factors, Lombroso drew up a so-called "food section", which comprised diseases that arise from the misuse of specific foods (zea maiz of cactus opuntia and latyrus sativus) or drinks. Finally, he created an "ethnic section", in which the forms and species that acquire particular diseases were grouped according to the characteristics of the country's various populations.

Lombroso's observations corroborated the studies of Giuseppe Sormani who had drawn up thermometric and demographic curves. Sormani had plotted the data on births and deaths in 15 areas of Italy and compared these with those on temperature and rainfall recorded by meteorological observatories in the same areas (27).

Schiaparelli's demonstration of the effects of the moon on the barometer and on many other climatic characteristics led to the hypothesis that it could also influence the human organism in a more or less direct way (28) .

Meteorology books proved to be a valuable source of data, though the analysis of isothermal lines required caution, since the large averages were figures that told the truth in their own way, to a certain extent and under certain conditions, but could not always be used to explain real cases.

It should be remembered that meteorology had dealt not only with the seasons and changes in the weather, but also with the influence of the stars, as Giuseppe Toaldo had pointed out a hundred years earlier (29). These orientations were then found in *Pensiero e meteore* by Lombroso and colleagues. Cosmotelluric knowledge thus gained impetus in the medical sphere.

3. Conclusion

Attentive to the health and anthropological issues that constituted part of his research activities in the early years of his career, Lombroso tackled the grave shortcomings of health and social organization. He proposed public health interventions, such as draining swamps, canalizing rivers, promoting the cultivation of many lands, deforesting wooded areas near villages and roads, building ports, offering settlers better houses in the countryside and adopting a uniform system of sewers. Of course, as we have seen with regard to pellagra, we cannot ignore that some interpretations have also delayed economic-social reforms indispensable to the protection of the population health. Nevertheless, when we read those pages, we must bear in mind that they were written before the discovery of microbial agents, and it is not surprising that they contain many elements that would later reappear in the author's best known psychiatric studies. Lombroso grasped the important differences among the various localities and regions of Italy, and was fully aware of the shortcomings of public health and the need for hospitals built according to updated criteria.

All these factors had a certain importance in the political and social field, as they marked an unsurpassed limit by the liberal state with regard to interventions that acted on private interests and were the starting point for social action in the name of hygienic prophylaxis (30).

In the analysis of Lombroso's writings prejudices emerge against criminals, mads, primitive peoples and even women who are unacceptable and completely repudiated by science. However the theory of Cosmotellurism in Lombroso's work is not only a source of unquestionable interest in the history of medicine. The heritage of medical geography within the pre-bacteriological scientific culture, can in fact continue with its teachings to correctly address the clinician's thinking even in the current historical context in which, even after long periods of latency, endemic and epidemic pathologies re-emerge in various parts of the world.

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Original article

History of use and abuse of X-ray: the early 20th century Italian pediatrics school

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Summary. In our paper we report a brief history of the X-rays discovery and discuss the implications of their use and abuse in the Italian pedriatic schools of the early 20th century. Indeed, history of the X-ray treatment in the Italian Pediatric School has not yet been well studied. Even if the scientific experience of many physicians is well known in literature, a summary was missing. In Italy, in 1900, exposure to Röntgenand ultraviolet radiation or to large amounts of solar rays was a widespread medical practice, especially in several pediatric schools. During those years, diagnosis and treatment of childhood pathologies underwent considerable changes, especially after the twenties, when scientists developed an unquestionable trust in the therapeutic properties of radiation, considered harmless at that time. We report the main steps of the scientific research of the early 20th century in Italy. (www.actabiomedica.it)

Key words: pediatry, X-ray, 20th century, Italy, history

Introduction

X-rays are a form of electromagnetic radiation with a wavelength ranging from 0.01 to 10 nm (10^2 - 10^{-2} Å), thus significantly shorter with respect to visible radiation. They have good "hardness", which is the ability to penetrate deeply in opaque objects. The hardness depends on the wavelength of the X-rays and must be calibrated according to the thickness (t) and physical-chemical properties of the irradiated object. Radioscopy is an investigative technique, consisting of the impression of X-ray on platinum, zinc, cadmio or calcium-tungstate zone plates. Nowadays it is widely used during surgical interventions and in the guided fracture reduction, whereas it is not so common in other fields. The final imagines obtained with those techniques are less clean than radiographic ones.

Röntgen rays, most commonly known as X-rays, were discovered 8 November 1895 in Würzburg, in Germany, during some experiments with the Hittorf-Crookes tubes for the production of cathode ray. The experiments were carried out by W. C. Röntgen, a professor of physics at the University. The first X-ray radiography obtained by him, depicting his wife's right hand with a large ring in the middle finger, attracted much attention on the subject. A few months later, in 1896, Henry Becquerel discovered radioactivity, while working with phosphorescent materials. In December 1896 Pierre and Marie Curie, who actually coined the term radioactivity, conducted further pioneering

studies on radium and polonium isotopes. Although in 1901 Röntgen won the Nobel Prize in Physics he never patented his discovery. For this reason, in 1928 his colleagues named "Röntgen (R)" the unit of measurement for the exposure of X-rays. It is interesting to point out that X-rays were used soon also for the study of the mummies in order to comprehend "hidden pathologies" (1), practice still used today in the human remains (2, 3)

Starting from 1896, several cases several cases of visual system and skin diseases were reported. In the first years of the 20th century, the scientific community draw attention to the possible correlation between X-rays and leukemia. In order to enlighten the dangerous effects of X-rays exposure, the Anglo-American engineer Elihu Thompson irradiated his left pinkie for a week causing skin lesions.

Little was known on the phenomenon and there was the belief that the exposure to this kind of radiation could be helpful in the diagnosis and treatment of several diseases. Therefore, experimentswere conducted on patients of all ages and suffering from various diseases.The use and abuse of radiation was frequent among the operators, some of whom, voluntarily or more often inadvertently, exposedthemselves to the harmful effects of excessive doses of ionizing radiation (4). Operators used to work in over-exposed environments with rudimentary instrumentation, lacking in an adequate protection. Children, equipped with appropriate glasses could take advantage of the beneficial effects of ultraviolet radiation in specially prepared indoor environments. Exposure to sunlight was proposed in small degrees with the use of complex tables (5). Until 1939 there had been still the belief among physicians that the effects of unduly dosed sunlight and an outdoor life could cause lucite, a phatology directly connected with the onset of pellagra, erythema multiforme and lupus. The enthusiasm of the researchers for the newly discovered X-rays led them to underestimate the serious side effects that were outlined byphysicians in the early years of the application of this innovative technology. Serious eye and skin damage, and even leukemia, were reportedalready in 1896. In Europe, one of the first hospital using extensively X-rays was the "Royal London Hospital" and in 1898 the British surgeon John Hall-Edwards (1858-1926), a military doctor, politician and passionate photographer, had his left hand amputated (still preserved in the Birmingham Museum) due to severe dermatitis contracted during the first years of experimentation. In the 1920s, the harmful effects of X-rays on the genome were outlined.

Having raised awareness on the side effects of the use of ionizing radiation, there was the willingness to reduce as much as possible the exposure of the subjects: aluminum filters, collimators, darkest photographic plates and general good practices for insiders and patients were introduced in the first years of the 20th century, especially by the British Röngten Society (1915) and the American Röngten Ray Society (1922) (6).

In Italy, the first enthusiastic experiments on the use of X-rays were conductedin 1896 at the Military Hospital of the Trinity, in Naples, by the lieutenant colonel Giuseppe Alvaro, who made use of the new technique on injured soldiers from the war in Africa.

During the First World War special vehicles, called Radiological Ambulances, were born. These four-wheeled life-saving mobile X-ray units were supplied to military hospitals for the emergency care on the battlefield.

The use of radiology was also reported at the end of the 19th century in neuro surgical departments and in psychiatric hospitals (7). Colonel Alvaro extended the use of X-rays to military hospitals as an attempt to treat Tbc, Tifo, Colera (8). This practice became very common, and in the early 1900s, some authors reported the use of radiation therapy in severalacute inflammatory conditions such as lymphadenitis, boils, paterecci and other inflammatory skin conditions. Radiation therapy gave good results in the treatment of Tinea capitis, a pathology that afflicted mostly pre-puberal children from deprived social environments.The Radioterapic Institute was established in 1914 under the direction of Prof. Francesco Radaelli and was attached to the Dermosifilopatic Clinic of Cagliari. In its first year of activity in Cagliari they carried out thousands of treatments, the most numerous with the radion emitted by radium and Röntgen rays, on diseases such as Lupus, epithelioma of skin and mucosa, tigne and onychomycosis. Radiation treatment was first praised as being effective, painless and practicable in the clinic but many years later it was reported that the irradia-

tion of the head or neck of children was the cause of numerous skin and brain neoplasia and thyroid cancer (4). An Italian method for the use of Röntgen rays in childhood cardiology was proposed by prof. Francesco Visco, (Pediatric Clinic of Naples)during the VII Italian Pediatric Congress held in Palermo between the 20th and 23rd of April 1911. In his presentation, Visco referred to the first work of Dr. SeverinoArnone in 1910 (9).

The experimental irradiation of ten pregnant women conducted in Germany in 1925 by J. Zappertinformed the italian physicians about the secondary damage of this practice for the mother and the baby, especially during the first trimester. The study of ten cases of pregnant women exposed to irradiation in Germany in 1925 allowed to outiline the secondary damage of this practice for the mother and the baby, especially during the first three months. To verify the harmlessness of irradiation before pregnancy, the Pediatric Clinic of Siena, directed by M. Pincherle,carried out an experiment and reported the outcomes (10). In the treatment of rickets, among pediatricians, it was common opinion that cow's milk, powdered milk, or other food or drugs, if properly irradiated, could have special therapeutic powers. In 1928, irradiation techniques of milk, liquid and powder were discussed in a well-known pediatric magazine appeared to have become common methods (11, 12). The new practice, appropriately and variously dosed, was used experimentally in pediatrics to irradiate nursing mothers.An experiment in air irradiation was conducted by Luigi Spolverini on ten mothers hospitalized in Paviawith the aimof increasing their milk secretion (13). Bread, wheat flour, olive oil, subjected to radiation, were given to rats by Spolverini in order to evaluate their eutrophic action. At the end of the experiment, the author concluded that to obtain the desired effect in young organismsit was necessary to make food adequately irradiated with skillful use (14). The eutrophic action of irradiated foods was experimented also by other authors (15). Average doses of radiation at close range on powdered milk and fresh cow's milk were used by the pediatrician Spolverini, with good results for the treatment of seven cases of rickets. The same author recommended higher doses of radiation in particularly serious cases. Interested in the physical changes produced by UV radiation on food and on bodies, the pediatrician Luigi Spolverinipublished more than three hundred scientific papers on this subject (16). As it is known, even if today rickets is mainly limited to genetic forms (hypophosphatemic, vitamin-D resistant, renal tubular acidosis), a nutritional rickets could be developed in immigrated children with dark skin (17). Ultraviolet rays were used experimentally from 1923 to 1926 in the therapy of hypocalcemic rickets in 20 children between the ages of six months and three and a half years. The children, encouraged to live as much as possible in the open air, were irradiated with lamps of various kind, in sessions three times a week or every other day, for 40-60 minutes, in the anterior and posterior regions of the body. The relationship between clinical symptomatology and humoral relief was not constant but the results achieved on the reduction of tetany were considered satisfactory by the authors (18).Several French authors observed that the radiation therapy (splenic and hiliar) had given inexplicable nonhomogeneous results in the treatment of 64 children affected by asthma and spasmodic coritza (19). The second International Congress of Pediatrics (Stockholm 18th-21st August 1930) was an opportunity to discuss and exchange views among pediatricians all over the world on the properties and beneficial effects of UV radiation and radiated ergosterinfor the wet nurses, in order to prevent and curerickets, tetany and osteomalacia. In the introductory report of Adolf Hess, in favor of these therapies, he warned the audience about an indiscriminate use,without scientific basis, of UV and irradiated products, some of which, like ergosterin, had still obscure mechanisms of action (20). In 1947, at the XVIII Italian Congress of Pediatrics, curated by OrazioMalaguzzi Valeri, the theory on the relationship between spring solar radiations and stunted manifestations, such as tetany, was presented. The lack of ultraviolet radiation was identified as the cause of hypophosphatemia or of rickets, but for some doctors these diseases were connected with food or vitamin deficiency. Tetanus crises were more frequent in spring, but the intimate relationship between spasmophilia and vitamin D deficiency and UV rays was quite clear to academics.The studies conducted byFrontaliin Italy, revealed how sunlight had the power to mobilize phosphate ion deputies in the blood stream, with un-

known modalities, in order to fix calcium to bones (21). In 1929, G. Careddu, a young assistant of prof. Gino Frontali, director of the Pediatric Clinic of Cagliari, described in his work"Infantile Splenic Anemia and Actinic therapy", appeared inthe Journal of Pediatric Clinic, the use of irradiation in twelve cases of mediterranean anemia in Sardinia.

For an unknown period, the children who arrived in Pediatric Clinic underwentan experimental actinic cure with irradiations on all of their body, supplied at a distance of sixty cm and with increasing duration from a 150V Hanau mercury vapor lamp (podium presentation: Maria Francesca Vardeu, Storia della beta talassemia in Sardegna, Convegno di Storia della Medicina Bergamo e la Sardegna: due "isole" gemelle. Punti d'incontro e analogie attraverso argomenti di storia della medicina. Bergamo, Sala Mosaico della Camera di commercio, 1-2 dicembre 2007) il contributo delle scuole italiane di Pediatria). Mercury-vapor lamps (22) were usually used in houses in the past for they brilliance, even if today they are in disuse also in external environment due to their toxicity. The vapours, contained in a glass or quartz tube, were ionized by a stream of electrons and ions produced by two electrodes, giving a brilliance mainly in the ultraviolet radiation. The exterior part of the lamp is completely covered by a fluorescent powder (yttrium vanadate or yttrium aluminate) transforming ultraviolet radiation in radiation in the visible spectrum (Exemples of this instrumentation can be consulted here: Storia dell'ISS: Collezione di strumenti dell' ISS, Archivio Fotografico, www.iss.it). Already in 1911, Petrone and Lo Re reported that the first therapeutic treatment with Röntgen rays of infantile splenomegalies with anemia was performed by the Roman paediatrician Luigi Concetti on a three-year-old boy who came to his observation in the early 1900s. The case was presented at the Congress of Pediatrics held in Rome in 1905 where Concetti (23) said that after twenty-five irradiations, the spleen tumor had decreased considerably, but, unfortunately, anemia and systemic toxicosis appeared in the child. In 1939, Tecilazic, at the XVII Italian Congress of Pediatrics, referring to Cooley's anemia therapy, stated that all the attempts made to decrease hyperplasia of erythropoietic tissue, by irradiating the bones and spleen with Roentgen rays,had given negative results. Ultraviolet radiation or Rontgen rays were applied to children for the treatment of lymphatism, and in adenoidism, directly or by inhalation, with nebulized and irradiated drug solutions. It was thought that the radiation had a beneficial effect in the treatment of pertussis, mumps, and infantile atrophic diseases or in all cases of endocrine hypofunction. Roentgen rays were frequently used in common diseases of childhood, but also in vulvovaginitis, in the treatment of meningococcal and tuberculous meningitis and in the treatment ofpost poliosyndrome. In 1938,irradiation of the spleen was still practiced as a preventive hemostatic procedure in the surgical preparation of tonsillectomies. The Nasopharyngeal cord of patients with diphtheria bacilli was "sterilized" with a local application of X-rays. This method was still consideredsafe in 1939 by the president of SIP, the pediatrician G.B.Allaria.A detailed and significant radiological case study was conducted on children affected by mediterranean anemia and carried out by the Pediatric Clinic of Bologna(directed by Maurizio Pincherle)and the Bologna Orthopedic Clinic (directed by Valerio Putti). It was published in 1938 in an important pediatric journaland proposed the somatic criteria that were used for the identification of the Cooley anemia in the Italians children (24-32).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Lithographic lecture notes. A tool of forensic medicine teaching. Observation on the lessons of Paolo Pellacani (1884-1885), forensic physician at the University of Pavia

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Summary. We focus our attention on the use of lithographed lecture notes written by professors, or more often by students, in the teaching of medicine and surgery courses, between the late 19th and early 20th centuries, a period in which, to better understand the phenomena underlying life and death, collaboration between medical professionals and natural science researchers was intense (1). In particular, we analyzed the lithographed lecture notes of Professor Paolo Pellacani at the University of Pavia for the course of legal medicine. (www.actabiomedica.it)

Key words: medical education, legal medicine, Paolo Pellacani

Introduction

We reflect on a teaching method used in the late nineteenth century in Italian universities, when lessons printed in the form of "lithographic lesson notes" were offered to teachers and students as an economic tool, able to integrate or replace the published scientific manuals and treatises. This is a topic little studied by medical historiography and the opportunity to enter specifically in the subject was given to us by a small collection of some books and medical manuscripts that had belonged to Alessandro Achille Tettamanti (1859-1931). Today they are stored in the Civic Library of Varese. We here focus our attention on the text containing the Forensic Medicine lessons taught by Paolo Pellacani (1854-1920), professor in Pavia (2). These lithographed lecture notes are bound together with the free course of gynecology held by Alessandro Cuzzi (1849-1895) in the same school year (1884-85) and collected by Cesare Lampugnani.

This lithographic lesson text, collected and transcribed by the students R. Fusari and E. Cesari, measuring 24,5 cm in height, titled "*Lezioni di Medicina legale del Prof. Pellacani raccolte da R. Fusari ed E. Cesari l'anno 1884-85*", and divided into 15 lecture notes for a total of 117 pages, was used by students of the Faculty of Medicine of the University of Pavia. The last page carries the date of June 8, 1885, to be understood as the last day of the course.

We know that, after the Magister at Pavia, also in other universities in which Pellacani taught, lithographed lecture notes of his lessons were drafted (3,4).

In Bologna, at the end of the century, there were notes from Pellacani's medical courses which had been collected by Giulio Obici (*Lezioni di Medicina Legale date nell'Università di Bologna nell'anno scolastico 1892-93 e raccolte da Giulio Obici. Parte generale*, Bologna, Lib. Fratelli Treves di Pietro Virano, 1893, 114 pagine con 29 figure), additional notes were collected by Giuseppe Zamboni in the year 1899-1900 (165 pages).

There are also lithographed lecture notes by Pellacani for students of law, and we know about those concerning the years 1897-1898 (94 pages) and 1899-1900 (66 pages) and those collected by the student Gi-

useppe Osti in 1903-1904 (153 pages). Only in 1915 the professor published the first volume of his "*Notions of forensic medicine in summary*" (in fact the only one published in Bologna at the Salesian printing school, 316 pages).

The lithographic lecture notes

It should be noted that the lithographed lessons were generally used as teaching tools in universities over the decades between the nineteenth and twentieth centuries. We know of lithographed lecture notes for many of the subjects for teaching medicine (5) and they were very popular among students, replacing manuals and treaties for the purpose of education and examinations.

However, today, these volumes are difficult to find in libraries. As a matter of fact, due to editorial modesty and their low value, they were rarely preserved and are often in bad condition.

This is a pity as these volumes represent, in many cases, one of the most important tool to really know - with the precise transcription of the words used by the teacher in the classroom - the modalities and contents of teaching by many professors. The technical panorama of the time had allowed the use of this modality to spread because only a limited number of professors gave the press summary volumes of their specialized knowledge for educational purposes. Therefore, if one wonders what circumstances may have encouraged the development of lithographed lesson notes for forensic medicine, the answer could possibly come from the careful examination of the manuals and treatises present in the libraries of that time. In the second half of the nineteenth century medicine had already embraced the experimental method of positive science, so that even for forensic medicine treaties of a few decades earlier could be partially overcome.

In Pavia, were still circulating volumes of the *Fundamentals of Analytical Forensic Medicine* (1852-1854) by professor Giovanni Gandolfi, who had held chair from 1866 until 1875. It is this kind of exposure that is hinged on classical models of tradition. Moreover, even in other universities, when it was necessary to update specialist literature, it suffered from delays and there were never enough new textbooks available in bookshops.

It's interesting to note that the production of medical-legal manuals, at that time, was more frequent at the University of Naples where the work of Giuseppe Ziino and Luigi De Crecchio appeared in the seventies and eighties, as well as the Italian version of the treatise by Alfred Swaine Taylor). In Naples, the *Legal Medicine News* of Vincenzo Giovene and the *Practical Guide to the civil and criminal legal medical reports* of Antonio Raffaele were published. A Neapolitan printer also became involved in the Italian translation of the monumental German treatise on forensic medicine edited by Joseph Maschka. This localized and flourishing interest may not be a surprise when we consider the strong tradition of legal studies in the Neapolitan University.

In the north of Italy, the outlook was not as bright: Giuseppe Lazzaretti, a professor in Padua, published up to 1880 updated editions of his treatise (*Forensic medicine, a rational method for solving civil and crimi-*

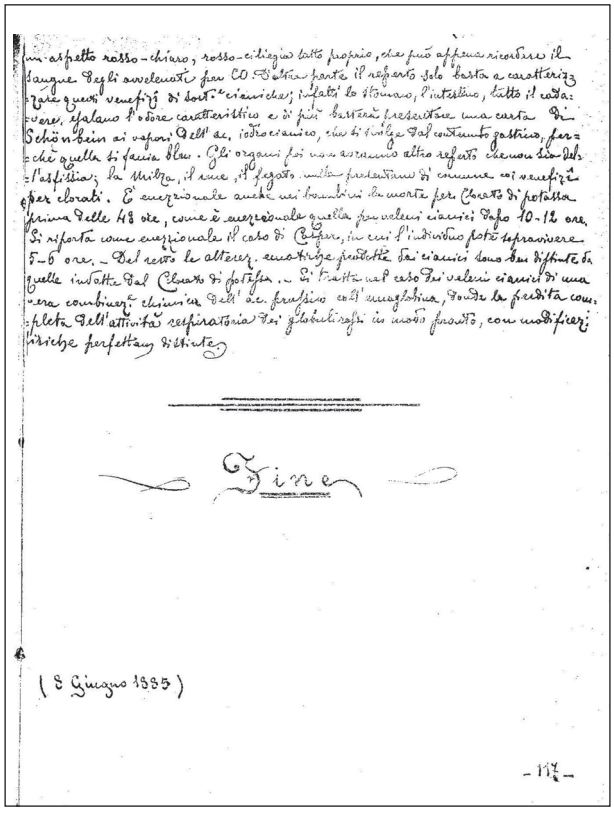
Ziino

Figure 1.

nal matters) that had first appeared in 1853. According to Laura, in charge of teaching in Turin for a year in 1873, he printed his *Treaty of Legal Medicine* in 1874, but three years prior to this his lessons had already appeared. The major foreign authors were also circulating in Italy, and among the French, at that moment, the Handbook (Naples 1853-1854) of Joseph Briand and Ernst Chaudè remained very popular, and above all the well-known work of Alexandre Lacassagne, which was translated into Italian in the early twentieth century. Perhaps the Italian edition of the Practical manual by Johann Ludwig Casper, who was also an author often cited by Pellicani, still made sense at that time. Furthermore, in this period, there was the question of the legislative update of the country that had inherited the civil and criminal codes of the old states; this update was not irrelevant for legal doctors, who were very interested on the discussion concerning the new legislation, which, finally arrived at the fundamental stage of the Zanardelli code (6, 7).

Considerations

Pellacani's scientific production had been particularly rich in and demonstrative of the specific interest that we partly find in the lithographed lessons we are dealing with today. In scrolling through these pages, it emerges how the professor presented himself to his students : he always demonstrated a secure approach based on deep knowledge not only in the theoretical part but even in the practical exercise of the discipline.

Lithographed lecture notes are unambiguous documents of all the course content given by the professors and therefore offer an authentic testimony of how the lectures took place during the course of an annual program. We can assume that they are demonstrating the major, or more specific, interests of the teacher, or the lines of practical commitment that represent the decisions made by the professor in the classroom. We can see that the course had been articulated well in some chapters, which, however, did not exhaust all the topics of the discipline, but it is not our intention, here, to go into the details of all the chapters. Pellacani had been quite critical about the problem of the discipline in Italy and his lessons were demonstrative of the attention he wanted to pay on the practical part and on the exercises (7). In a systematic and clear way, he explained what he thought to be necessary to guide physicians in the field of law, putting precise questions to which he responded with precise answers. Reading the text we can notice that his expressive modalities did not highlight those asperities that had been witnessed by Raffaele Guerrieri, his assistant in Bologna, who remembered the difficult character of the professor, who changed 18 assistants between 1891 and 1920, and consequently lived almost isolated. To explain the concrete applicability of forensic medicine, in addition to the continuous citations of the articles of the penal code concerning the various topics, there was no lack of listing the possible questions in criminal cases to which the doctor had to answer, to assert the arguments in the Court. Pellacani entered the formal part of forensic medicine by placing the students in front of the first question: the coroner is a witness and like any other professional, he must lend his work if legitimately called upon. On this, the code of law then in force was absolutely clear. He recalled students of their duty to comply with reporting cases of judicial interest, citing the risks arising from inobservance, including suspension from the profession or even criminal penalties.

The teacher was quite precise when detailing how to notify authorities with anything that might be of interest and how to draw up a legal-medical notification in all its various parts: preamble, stating facts, discussion, judgment, conclusion, avoiding all that could give rise to ambiguity or interpretive contentions. We can see that, if the professor insisted on these aspects and on the responsibilities of the physician obliged to the crime notification, in all his lessons the risk of being accused for lack of skill or imprudence in the exercise of the profession was however never mentioned.

Didactics dealt with the theme of personal injury and suspected poisoning, circumstances that obliged to inform the authority. So a large part of the course was reserved for exposing the so-called personal injuries with a detailed discussion of the damaging agents: usual and unusual weapons. Emphasizing the difficulties inherent in the evaluation of dating the injury or the precise determination of the weapon used, he listed the aggravating circumstances with the presentation of

concrete evaluation examples, accompanied by the issues of differential diagnosis emerging from time to time; he explained the forensic concepts of organ, danger of life, functional impairment, consequences of the injuries at work and the permanent disfigurement of the face, at a time in which crime involving disfigurement was frequent.

The bodies of crime included blood, which, when appropriately examined, offered many subjects of study. The analyses required to carry out an accurate search for blood in suspect areas were illustrated.

Another frequent causes of violent death was asphyxiation, of which were presented the common characteristics, in addition to the anatomical-pathological picture resulting through external and internal examination of the corpse.

Once the formal part was finished, the professor proposed some casuistic exercises concerning infanticide or procured abortion also recalling the most frequent questions asked by the judge in this regard. Finally we note that the most substantial part of the lesson notes is formed by themes in the toxicological field, which seems to have been dominant in Pellacani's interests.

We can notice that aspects of tanatochronology had been completely neglected, whereas, as we know, they appear as one of the principal topics in his scientific production, in harmony with the teaching of his teacher Arrigo Tamassia.

Evidently to the student Pellacani offered a summary presentation, useful to a generic professional, while when it was necessary to deal with legal cases he already highlighted the need for special medico-legal skills and, like other university colleagues, he asked aloud the courts to entrust the task of experts only to physicians specifically prepared in medical-forensic matters.

Anyway not all arguments of forensic medicine were presented and some topics that could not be ignored by the students were excluded. This suggests that even if the professor restricted the field of exposure from the chair to what we can read in the lithographed lectures, for the exam preparation it was likely necessary for the student to equip himself with treaties or manuals of scientific publishing.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

Post-amputation neuroma of radial nerve in a patient with ephitelioid sarcoma: case report and literature review

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Summary. Neuroma, also known as traumatic neuroma or amputation neuroma or stump neuroma, is a focal non neoplastic area of proliferative hyperplastic reaction secondary to peripheral nerve damage that commonly occurs after a focal trauma (acute or chronic) or surgery, such as amputation or partial transection. Neuromas are more commonly located in the lower limbs, followed by head and neck; other extremely rare sites include the ulnar nerve followed by the radial nerve and the brachial plexus. A radiologic plan is necessary to recognize soft tissue lesions with a neural origin and whether they are a true tumor or a pseudotumor such as a neuroma, fibrolipoma, or peripheral nerve sheath ganglion. In oncologic patients the appearance of post-surgical neuromas can produce problems in differential diagnosis with local recurrences. Therefore, with a combination of different imaging techniques, mainly ultrasound (US) and magnetic resonance imaging (MRI), it is possible to characterize neurogenic tumours safely, with a great impact on patient management and to plan an appropriate treatment. Here, we report the first case of post-amputation neuroma of radial nerve in a patient with clinical history of ephitelioid sarcoma with a short literature review. (www.actabiomedica.it)

Key words: neuroma, peripheral nerves, tumors, sarcoma;, ultrasound (US), magnetic resonance imaging (MRI)

Introduction

Neuroma is a rare benign lesion affecting peripheral nerves characterized by nonneoplastic proliferation of the proximal end of a partially or completely transected nerve (1). It can be classified into two categories: terminal neuromas and spindle neuromas (2). The most common presenting symptom is pain, numbness, discomfort or electric shock-like symptoms, but approximately 20% to 30% of all neuromas are painful (3, 4). Neuromas are more commonly located in the lower limbs, followed by head and neck; other extremely rare sites include the ulnar nerve followed by the radial nerve and the brachial plexus (3).

An early accurate diagnosis and appropriate treatment are crucial for a good outcome. A radiologic plan is necessary to recognize soft tissue lesions with a neural origin and whether they are a true tumor or a pseudotumor such as a neuroma, fibrolipoma, or peripheral nerve sheath ganglion (1). Imaging techniques such as ultrasound (US) and magnetic resonance (MRI) imaging are the best modalities to characterize these lesions and have a direct impact on correct management and treatment of patients (1). Here, we report the first case of post-amputation neuroma of radial nerve in a patient with clinical history of ephitelioid sarcoma with a short literature review.

Case report

A 34-years-old man, with a clinical history of excision of a soft tissue mass at the right forearm for epithelioid sarcoma, comes to our department for the appearance of a palpable nodule at the region of the right wrist. The patient reported discomfort at focal pressure at the nodule site. The clinical examination showed a mass of soft tissue with pain on palpation, Tinel's sign positive and there wasn't sign of inflammation.

A search for recurrence of ephitelioid sarcoma was undertaken. Thus, conventional B-mode US, color and power-Doppler (CD and PD) and sonoelastography examination of the right forearm were performed.

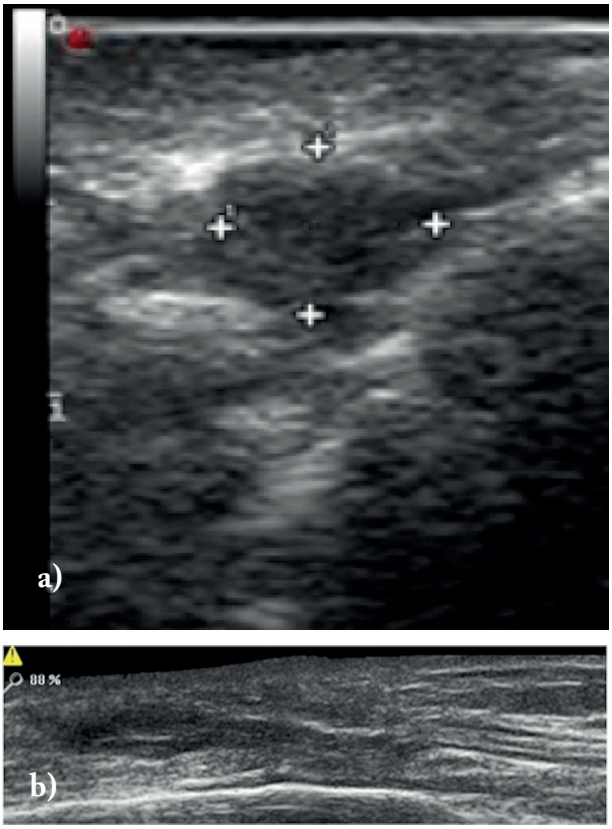

Figure 1. US B-Mode axial view (**a**), longitudinal view (**b**): marginated homogeneously hypoechoic fusiform mass with echogenic strands inside and a "bulbous end" morphology appearing to be in continuity with a normal nerve proximally. In longitudinal US B-mode scan we evaluated clearly the hypoechoic nerve entering in the ovalar mass, which didn't infiltrate the muscular fascia. We studied also the radial nerve along its whole course where the nerve echostructure was normal and with stable size

B-mode US showed a marginated homogeneously hypoechoic fusiform mass with echogenic strands inside and "bulbous end" morphology appearing to be in continuity with a normal nerve proximally (Fig 1). In longitudinal B-mode scan we evaluated clearly the hypoechoic nerve entering in the ovalar mass, which didn't infiltrate the muscular fascia. We studied also the radial nerve along its whole course where the nerve echostructure was normal and with stable size. On CD examination, few vascular signals inside and around the radial nerve were detected (Fig 2a).

US Strain Elastography (USSE) was performed with the patient lying in the some position as for B-mode scanning by applying a light compressione with the US probe. The US elastogram was displayed over the B-mode image in a color scale depending on the magnitude of strain: red (soft tissue), green (intermediate degree of stiffness), and blue (hard, anelastic

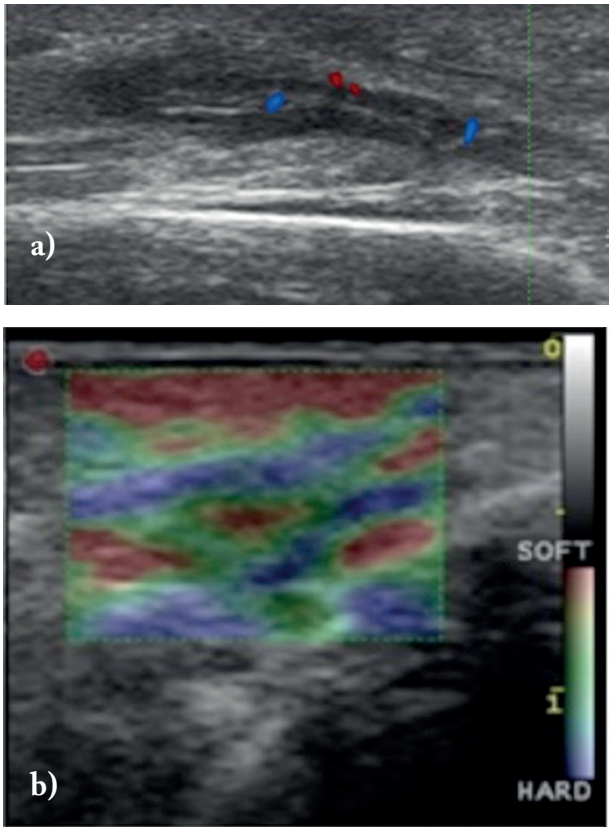

Figure 2. (**a**) Color-Doppler US (longitudinal view): few vascular signals inside and around the radial nerve. (**b**) US-elastography (axial view): the soft tissue nodule showed elasticity in the whole area with a contextual small mixed red area inside

tissue). The soft tissue nodule showed elasticity in the whole area with a contextual small mixed red area inside (Fig 2b).

MRI examination was further performed with a 1.5 tesla resonance magnetic imager (Symphony – Siemens. Erlangen), which showed a well-definited ovoid subcutaneous lesion with an intermediate signal intensity (similar to that of muscle) on T1-weighted images and an intermediate-high signal intensity with a typical fascicular pattern on FSE T2-weighted images (with and without fat-saturation) (Fig 3). Histopathological examination, after excision, showed the definite diagnosis of amputation neuroma of radial nerve.

Discussion

Epithelioid sarcoma (ES) is a rare, high-grade malignancy that represents the most common primary soft tissue sarcoma of the hand (5, 6). ES predominantly affects young adults in their second or third decade of life, but may occur at any age; males are disproportionately affected with a ratio approaching 2:1 (5, 6).

Local recurrence often occurs within 1 to 2 years of treatment, and these patients often proceed to develop distant metastasis (5, 6). Radical tumor excision is the primary treatment for patients with ES (5). Therefore, differentiating between benign and malignant masses is important to prevent delays in the treat-

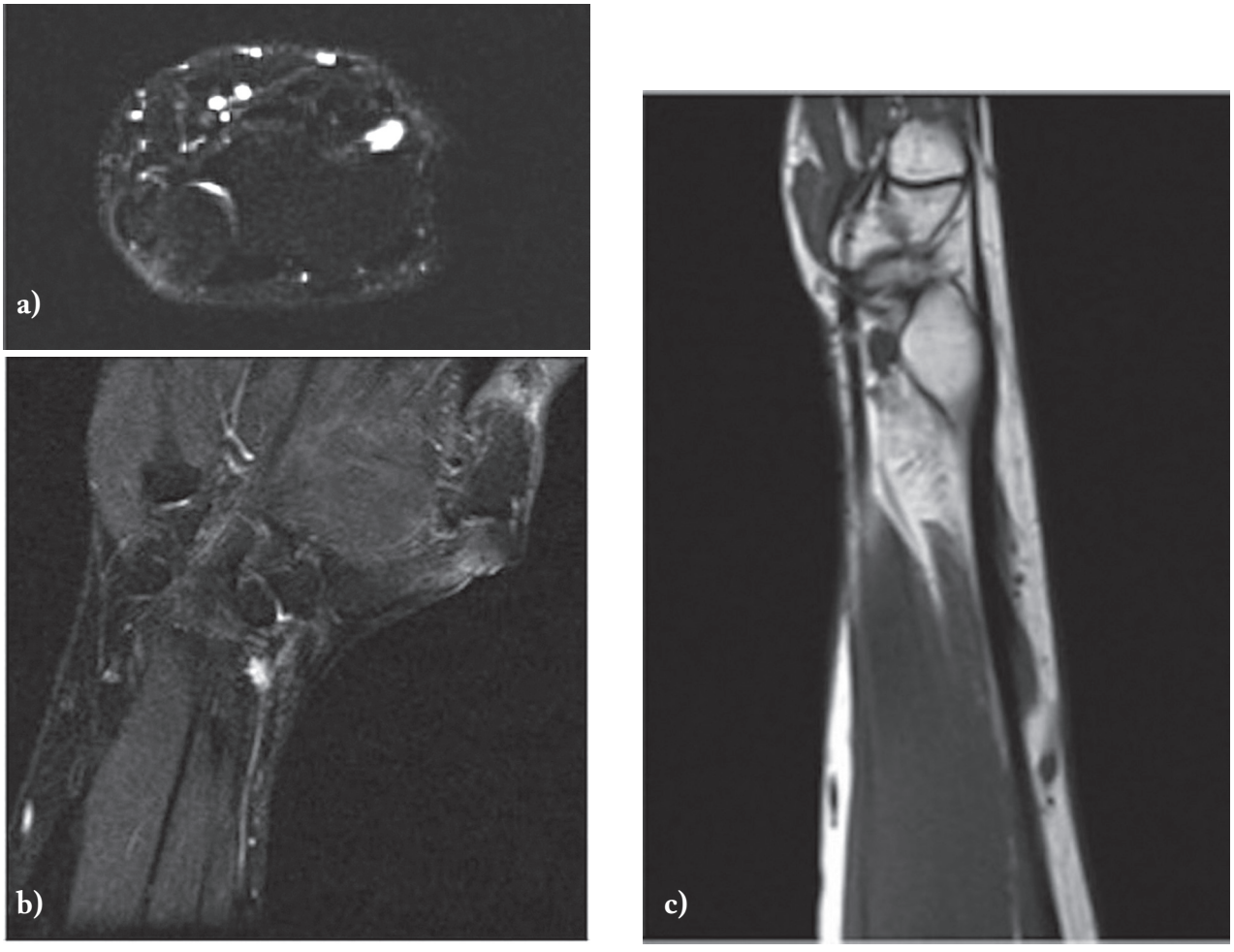

Figure 3. (**a**) FSE T2-w Fat-Sat axial (**a**) and coronal (**b**), T1-weighted sagittal sagittal (**c**): well-definited ovoid subcutaneous mass with an intermediate signal intensity (similar to that of muscle) on T1-weighted images and an intermediate-high signal intensity with a typical fascicular pattern on FSE T2-weighted images

ment of the malignant masses and avoid unnecessary surgical treatments for the benign masses.

Neuroma, also known as traumatic neuroma or amputation neuroma or stump neuroma, is a rare benign lesion affecting peripheral nerves and classified in the sub-category of pseudotumors from the World Health Organization classification of main peripheral nerve sheath tumors and considered in the scientific literature as purely benign lesions (7). Neuroma is a focal non neoplastic area of proliferative hyperplastic reaction secondary to peripheral nerve damage that commonly occurs after a focal trauma (acute or chronic) or surgery, such as amputation or partial transection (3). It is usually seen between 1 month and 12 months after injury but in literature cases have been reported as 8 days, or as late as 40 years (8). Neuromas are more commonly located in the lower limbs, followed by head and neck; other extremely rare sites include the ulnar nerve followed by the radial nerve and the brachial plexus (3).

Histologically, neuromas are composed of multiple and non-encapsulated axons, Schwann cells, endoneurial cells, and perineurial cells, surrounded by prominent scar tissue with dense collagen; dystrophic calcifications are rarely seen in the lesional area (3, 4). Disorganization of the neurogenic tissue (caused by multidirectional proliferation of cells in an abortive attempt to repair the injured nerve) allows traumatic neuromas to be distinguished from neurofibroma (9).

Two types of traumatic neuromas have been described, spindle neuromas and terminal (also called lateral) neuromas (3, 10).

Terminal neuroma originates at the end of the severed nerve and it is usually due to the proliferation of axons in any direction without the support of the Schwann cells in an abortive attempt to repair the nerve (3, 10). Terminal neuroma represents a normal pattern of healing of the nerve and is often asymptomatic (3). Spindle neuroma currently is considered as resulting from chronic stimulation and friction and it is localized in the nerve away from the severed nerve ending and represents the response of a peripheral nerve subjected to microtrauma due to stretching or compression by the localized scar tissue (3, 10).

An early accurate diagnosis and appropriate treatment are crucial for a good outcome (1). A radiologic plan is necessary to recognize soft tissue lesions with a neural origin, their association with a peripheral nerve, and whether they are a true tumor or a pseudotumor such as a neuroma, fibrolipoma, or peripheral nerve sheath ganglion (1, 11).

Imaging techniques such as ultrasound (US) and magnetic resonance (MRI) imaging are the best modalities to characterize these lesions and have a direct impact on correct management and treatment of patients (1, 11). In addition, US can be used to guide biopsy in difficult and uncertain cases when the lesion is either indeterminate or there is concern that the lesion is malignant (12).

Following the guidelines regarding soft tissue tumors in adults approved by the European Society of Musculoskeletal Radiology (ESSR) for peripheral nerve tumors, biopsy could be avoided in cases of purely benign lesions (1, 12).

US is the primary examination method for superficial soft tissue masses to confirm their size, location, the borders of the tissue masses, internal echo characteristics, internal blood low signal and association between the masses and the surrounding structures (13, 14). US offering high-resolution imaging, is quick and easy to perform and can assess vascularity that eliminates having to administer MRI contrast, saving costs and avoiding potential complications (14).

CD and PD techniques are a simple, non-invasive method able to increase the specificity of US by providing a real-time evaluation of vascularity, which is an important clue in distinguishing benign from malignant lesions; malignant tumors show an increased number of vessels, which generally appear distorted and deformed, and multiple peripheral poles (15).

According to the literature, US features such as well marginated hypoechoic ovalar masses with echogenic strands (fascicular pattern) in direct continuity with the radial nerve allowed to establish the possible neural origin of these soft tissue masses, specifically the neuromas (16). Other tumours of neural origin have been reported to be hypoechoic, e.g. neurofibromas and schwannomas, but these frequently were also associated with acoustic enhancement simulating cysts, which was not present in our case (16).

More recently, US contrast agents and US-elastography have been proposed as additional diagnostic

tools for the evaluation of various organs including soft tissue masses (17).

Stiffness of the tissue structures may be accessed using ultrasound strain elastography (USSE) (18). By applying pressure to the inspection sites, USE acquires response information resulting from the pressure and determines the tissue stiffness (18). The two most frequently used USSE methods are SE and shear wave elastography (SWE). SE acquires the deformation information of the tissues under pressure, with greater deformations indicating lower tissue stiffness and less deformations representing greater tissue stiffness and presents the results in different colors or differing degrees of brightness (18). SWE obtains the shear wave information from the tissues under pressure, with faster propagation velocities of shear wave indicating greater tissue stiffness, and also presents the results in different colors or differing degrees of brightness (18). In addition, SWE also measures and quantifies the shear wave propagation velocities at the regions of interest, and therefore provides more information compared with SE (18). Stiffness of a malignant tumor is typically higher compared with a benign tumor (17).

USSE has been widely accepted as an effective method for differentiating between malignant and benign tumors. Although differentiation of malignant and benign soft tissue masses using USE has rarely been investigated, some current studies aimed to assess the importance of strain elastography (SE) for the differentiation of malignant and benign soft tissue masses (18). However, to date, no case of neuroma studied with USSE is reported in the literature.

Contrast-enhanced ultrasound (CEUS) using second-generation contrast agents is a "new" simple, immediate, and effective diagnostic tool: microbubbles circulate freely inside the body and constitute an intravascular contrast agent; therefore, they permit analysis of both macro- and microvascular blood flow (19-22). To date, the value of CEUS for the examination of superficial lesions has not been studied in detail. CEUS previous studies of soft-tissue tumors demonstrated that microbubble-enhanced US can improve the detection of perfusion if compared with CPD (15, 23): in a substantial number of tumors that appear only sparsely vascularized with CPD, CEUS gives a completely different impression by depicting more vessels and more intense perfusion (15). Therefore, with these results, our group is planning to evaluate tumor neovasculature with CEUS.

MRI is the gold standard for superficial and deep lesions. Utilizing MRI it is possible to characterize the lesions: size, intratumoral lobulations, morphology, margins (well or ill defined) and detect perilesional edema, cystic changes (necrosis), interval change in size, vascularity, and effects on sur- rounding structures (1). The benefits of MR imaging are: less operator dependent, can assess deep structures more confidently, can cover a larger body part (whole-body MRI), can assess for skip lesions that often occur a long the nerve (14). However, the potential downside of MRI is that is relatively more time consuming and less available than US (1).

US and MRI are commonly used alone or in combination to study peripheral nerves (1). Overall, US is well suited for very small and superficial structures, whereas MRI is good for deeper lesion such as those located deep in the brachial plexus, lumbar plexus, and in patients with a high body mass index (1, 11). US is often the imaging modality initially used to study peripheral nerves in centers where both US and MRI are available (1, 11).

Conclusion

To conclude, we can assert that in oncologic patients treated for soft tissue sarcomas, the appearance of post-surgical neuromas can produce problems in differential diagnosis with local recurrences. Therefore, with a combination of different imaging techniques, mainly US and MRI, it is possible to characterize neurogenic tumours safely, with a great impact on patient management and to plan an appropriate treatment.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

An unusual cause of Steven-Johnson Syndrome

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Summary. SJS is a rare mucocutaneous syndrome characterized by skin and mucous detachment. The main etiological factors are drugs and infections; sometimes the cause remains unknown. In the prodromal phase we observed non-specific symptoms, followed by mucocutaneous manifestation. Due to risk of complications and mortality a multidisciplinary approach is needed. We present a case of a girl with an atypical presentation of SJS related to Enterovirus. (www.actabiomedica.it)

Key words: Steven-Johnson Syndrome, allergy, enterovirus, corticosteroids, cyclosporine A

Introduction

The Steven-Johnson Syndrome (SJS) is a potentially lethal acute mucocutaneous syndrome characterized by erythematous maculae with development of central necrosis, bullous lesions, followed by painful dermo-epidermal detachment with a frequency up to two cases per million every year, 10-20% of which in pediatric ages (1-3).

The cutaneous and mucosal manifestations are preceded by non-specific symptoms such as fever, rhinitis, headache, conjunctivitis, sore throat lasting approximately one week. Complication are infections, eye involvement (potentially leading to blindness) (2), scars that involve mouth, pharynx, esophagus, rectus, middle airways, genitourinary tract (phimosis, vaginal stenosis, dysuria) (4). Patients may develop renal and/or hepatic failure, dehydration, and sepsis.

The most common triggers are drugs, followed by infections and idiopathic.

The pathogenesis is not completely clear. However, SJS should be caused by a lymphocytotoxic response resulting in apoptosis of keratinocytes. The diagnosis is based on clinical findings, possibly supported by histology showing full thickness necrosis of keratinocytes in the absence of antibody deposits.

We describe a girl who had an unusual presentation and uncommon etiology of SJS.

Case report

A 14 years old girl went to the A&E because she was suffering for the last 3 days from rhinoconjunctivitis, edema of the lips and sore throat that developed after a day spent at the stable. She had a history of rhinoconjunctivitis and asthma due to grass allergy. She didn't take any drugs during 8 weeks before the onset of symptoms (5).

An allergic reaction was suspected and methyl prednisone 40 mg I.M. and chlorphenamine maleate 10 mg I.M. were given.

Symptoms persisted and the following day she went to A&E of the local Hospital. The patient had normal vital parameters, edema of the lips, rhinoconjunctivitis and generalized reduction of vesicular murmurs.

She was treated with oral cetirizine and prednisone and inhaled salbutamol. The ophthalmologist prescribed antihistamine eye drops.

On the following day, the patient developed fever (38.2°C), seropurulent ocular secretion and crusted lips lesions with de-epithelialization of the oral mucosa. At the A&E, complete blood count showed neutrophilic leukocytosis with normal CRP. Aphthous stomatitis was diagnosed. Oral acyclovir and tobramycin eye drops were prescribed, and previous treatments were stopped.

After two days, the patient had been visited by GP who diagnosed bronchitis and prescribed second-generation cephalosporin. The day after the girl returned to A&E due to persistent fever, catarrhal cough, bilateral palpebral edema, conjunctival hyperemia, edema and painful de-epithelization of the lips with crusted lesions, hyperemia and painful de-epithelization of gums and palate, erythematous maculae on the chin and on the left hand. At auscultation she had vesicular breath sound with rare scattered rales. She had normal vital parameters. The girl was admitted at hospital with a diagnosis of SJS. She had normal complete blood count with increased CRP (51.3 mg/L). Chest X ray was normal. Intravenous fluids were administrated. Furthermore, a *Mycoplasma pneumoniae* infection was suspected and Azithromycin was given.

Serological test results for antibodies to *Mycoplasma pneumoniae* (Virion/Serion, Würzburg, Germany), EBV (Vidas®, bioMérieux, Marcy-l'Etoile, France), HSV 1-2 (DiaSorin S.p.A., Saluggia (VC), Italy), Coxsackievirus (Virion/Serion), Adenovirus (NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany) were negative. The nucleic acid amplification assay (AllplexTM respiratory assays, Seegene, Seoul, Korea), performed on the pharingeal swab, revealed the presence of Enterovirus RNA. The amplicon of a nested PCR targeting the Enterovirus VP1 gene was submitted to sequencing (TIB Molbiol s.r.l., Genoa, Italy) in order to type the virus: the obtained sequences (forward and reverse) were not univocal and did not allow the typing. The viral cultivation of the pharyngeal swab, performed according to standard procedures (6), did not lead to the strain isolation. Renal and hepatic functions were normal. During hospitalization, the girl presented worsening of ocular symptoms with appearance of pain, visual loss and diplopia in the median fields. Ocular pseudo membranes were removed. Lubricant eye drops, corticosteroid eye ointment and chloramphenicol eye ointment were given. At the same time, she developed skin erosions at both ankles, onset of hyperemia, edema and painful ulcerous lesions at genitals, palate and tongue.

The girl was treated with daily oral rinses with chlorhexidine, viscous lidocaine at oral cavity, daily washing with neutral detergents at external genitals.

Because of severe oral pain with inability to be fed and the discomfort at the genital level, a pain-relieving therapy with morphine hydrochloride was given for 5 days.

SCORTEN score (7) was about 0, so the girl did not need to be managed in an intensive care unit.

The patient resumed eating from the sixth day of hospitalization; also, genital, cutaneous and oral lesions progressively improved.

On the eleventh day of hospitalization the patient was dismissed and mild de-epithelialization at the dorsal surface of the tongue was still present.

After 1 month from discharge, an ophthalmological visit was performed, and recovery of the eye was observed.

Discussion

We have presented a girl with SJS associated with Enterovirus infection.

The most common triggers of SJS are drugs, in 53-95% of cases (carbamazepine, phenobarbital, phenytoin, erythromycin, cefotaxime, trimethoprim-sulfamethoxazole, cloxacillin, amoxicillin, allopurinol, NSAID), followed by infections in 5-31% of cases (*Mycoplasma pneumoniae*, *Group A β-haemolytic Streptococcus, Rickettsia*, *Mycobacterium*, Cytomegalovirus, Herpevirus, Coxsackievirus, Parvovirus, Influenzavirus) and idiopathic in 5-18% of cases (1-3, 8-10). Many tests are warranted to identify the cause of SJS. When all of them are performed, it may be possible that they would clarify the etiology of many SJS that would be classified as idiopathic. In our patient, we did not find any relation between drug intake and onset of SJS. Investigations reveled an Enterovirus infection. To our knowledge this is the first case of SJS probably due to Enterovirus infection. The diagnosis of SJS is based

on clinical features and it is difficult to be suspected in the prodromal phase due to the non-specificity of the symptoms (1-3).

During the phase of epidermal detachment, we have also excluded by clinical and laboratory findings DRESS, Kawasaki syndrome, Staphylococcical scalded skin syndrome, atopic erythrodermia, pseudolymphoma (1-3, 11, 12).

The therapeutic approach to be used for SJS is controversial. In our case, the girl was treated topically until complete resolution of ocular, genital and cutaneous lesions. Moreover, she received systemic antibiotic because of the risk of sepsis, intravenous fluid replacement because of losses and food refusal, and analgesic.

High dose of systemic corticosteroids may be useful at the beginning of the disease but increased the risk of sepsis and GI bleeding (13). No difference on mortality rates between corticosteroids and supportive care has been described (13-14). In our patient a 3-day course of systemic corticosteroid during initial phase of the disease was not beneficial. There are promising results regarding effectiveness of cyclosporine A in re-epithelialization and prevention of skin lesions. However, further studies are necessary to evaluate its efficacy and safety in pediatric age (15).

In conclusion, our report shows that there is a need of a reliable biomarker for identifying patients with SJS. Such marker should be determined in samples collected by a non-invasive, safe and fast to perform method such as the use of exhaled breath condensate (16). The rarity of SJS hampers trials on therapy. However, further studies are warranted to clarify whether there is an individual response to different drugs and what the optimal treatment plan of SJS beyond supportive care is.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

Rotigotine effect in prolonged disturbance of consciousness. Brief report of two cases

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Summary. Two patients with post-coma reactivation deficiency who showed a "dramatic" response to rotigotine therapy are described. They had suffered from prolonged coma due to lesions in the mesencephalic ventral tegmental area. The authors believe that rotigotine effect in these cases could be due to restoration of dopaminergic transmission in medial frontal areas previously "de-afferented" from the lesions. Some comatous patients may experience a prolonged difficulty in recovering a normal state of consciousness. This phenomenon may be due to dysfunction of amynergic activating pathways connecting brainstem to the frontal cerebral cortex. In particular, dysfunction of dopaminergic pathways from the mesencephalon to the frontal cortical areas may be responsible for clinical pictures characterized by preserved alertness and total loss of interactions with the surrounding environment; the so called "waking coma" cases. (www.actabiomedica.it)

Key words: disturbance of consciousness, akinetic mutism, dopamine, rotigotine

Case Report

A 65-year-old man arrived at the emergency room because of acute consciousness disturbance. TC of the brain showed a hemorrhage in the mesencephalic region. His conditions were very critical (GCS = 3), even if the breathing function was normal. Family members were informed of a probable bad outcome over the course of hours.The patient was admitted to intensive care unit and then, after a few days, to a normal care unit. He remained stable for several days. After 20 days he recovered a waking state and he was transferred to a rehabilitation department for post-comatose patients. At that time the neurological examination showed a clinical picture of "acinetic mutism": the patient was alert but he was'nt able to make any movement either spontaneous or on request and the verbal expression was impossible. Occasionally he was able to follow the examiner's movements with his eyes. Considering the location of the lesion, it was decided to start transdermal rotigotine 2 mg/24 h, a dosage to be increased to 4 mg/24 h in the following week. In the following days the patient began making spontaneous movements and progressively improved his interaction with the environment; he was then transferred to a neuromotor non-intensive rehabilitation unit and after about 4 months he came back to our outpatient clinic with a normal mental state and left-sided mild hemiparesis which however allowed him to walk independently. Up to now he remained stable in follow-up visits. The second case regard a 50-year-old woman, after a bereavement in the family, spent many days locked in home refusing visits and food. After a couple of weeks she was found at home in a serious confusional state with delusions. A diagnosis of psychotic episode was made and she was treated with neuroleptics drugs for a long time. Delusions improved but two months after neuroleptic withdrawal she developed a total inability to move and to talk as for a severe auto-activation deficiency: he remained motionless on the bed, eyes open, without any interaction with the environment. A brain MRI showed an area of signal hyperintensity in

the mesencephalic region, possibly as a result of previous vascular or metabolic injury. Based on the clinical picture and the site of the cerebral lesion, 2 mg/24 h transdermal rotigotine therapy was started. The patient improved after a few days with reappearance of spontaneous movements; She was dismissed and she gradually resumed her job. After about 8 months she was able to return to work on a regular schedule as an employee.

Discussion

Case 1 describes a case who showed lack of awakening from prolonged coma with a prolonged "minimal consciousness state". Case 2 describes a patient with a clinical state of "akynetic mutism", which could not be simply explained as a collateral effect of neuroleptic therapy. In both cases there was a mesencephalic lesion in the region of the ventral tegmental area. Dopaminergic pathways departing from this area are directed to basal forebrain and they are considered to play a fundamental role in finalized behavior (1 - 4). They are component pathways of the medial forebrain bundle (MFB) which projects to mesial frontal regions and is crucial for the self-activation behaviour (4). Treatment with rotigotine may have improved the condition of these patients restoring dopaminergic transmission in "deafferented" mesial frontal areas (5). Rotigotine treatment may be actually helpful in patients with delayed awakening from coma or prolonged self-activation deficiency with akynetic mutism (5). The presence of mesencephalic or nucleobasal lesions in these cases may suggest the indication to rotigotine treatment, but however, in our opinion, in cases like these, due to cerebral injuries of various origin (traumatic, vascular, hypoxic, etc) a trial of dopaminergic therapy with rotigotine could be performed because a dysfunction of the dopaminergic pathways in the MFB could occur even if there are no lesions demonstrated by the usual imaging techniques.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Case report

Anaphylactic cardiovascular collapse manifesting as myocardial infarction following salad consumption. A case of Kounis variant type I syndrome

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Summary. Anaphylactic cardiovascular events constitute an underrated cause of medical emergencies in hospitalized patients. Coronary arteries and myocardium are targeted by anaphylactic mediators leading to acute coronary syndrome and imminent cardiovascular collapse. Early diagnosis and high clinical suspicion are required to secure prompt life-saving treatment in these cases. However, physicians of both Cardiology and Internal Medicine Departments are not familiar with this condition. Recently, we diagnosed and treated a case of anaphylactic cardiovascular collapse manifesting as acute myocardial infarction following salad consumption. Notably, Kounis anaphylaxis-associated acute coronary syndrome is a rare cause of ST segment elevation myocardial infarction with normal or diseased coronary arteries. We recommend that Kounis syndrome and its variants should be taken into consideration in the differential diagnosis of ischemic heart disease in patients with signs of allergic reaction and/or medical history of previous allergic reactions, who experience acute coronary syndrome after exposure to certain environmental stimuli. (www.actabiomedica.it)

Key words: acute coronary syndromes, allergic myocardial infarction, Kounis syndrome

Introduction

Anaphylactic cardiovascular events constitute an underrated cause of medical emergencies in hospitalized patients. A growing body of evidence suggests that the myocardium and the coronary arteries are substantially targeted by anaphylactic mediators (1,2). In this context, anaphylaxis-associated acute coronary syndrome (ACS) and myocardial dysfunction in line with systemic detrimental effects may ultimately lead to cardiovascular collapse (1-3).

Despite advances in management of acute coronary syndromes, patients with an anaphylactic cardiovascular event may necessitate additional treatment with anti-allergic drugs on top of anti-ischemic and anti-thrombotic therapy (3). Importantly, physicians should recognize early this clinical entity to ensure prompt and effective life-saving treatment. Nevertheless, anaphylactic cardiovascular collapse is understated in medical literature and the incidence, optimal treatment and prognosis of this condition are not delineated (3). Here we describe a case of Kounis anaphylaxis-associated acute coronary syndrome.

Case report

A 62-year-old Caucasian woman, presented to our emergency department with loss of consciousness, approximately 20 minutes after the consumption of a green home-made salad that included vegetables, tomatoes and peppers. Before arriving at the hospital,

the patient complained of shortness of breath, dizziness, excessive sweating, generalized pruritus, erythema and vomiting. Her previous medical history was significant for essential hypertension under treatment with valsartan 160 mg, once a day; while she reported two previous episodes of mild allergic reaction in the last decade to unknown stimuli, for which she had not sought further medical consultation. The patient did not have any history of tobacco, alcohol or illicit drug use. None of her medication was recently initiated.

Physical examination revealed an ill-appearing patient with diaphoresis and cold extremities in mild confusion that could not answer questions and follow commands. Diffuse erythema was present, whereas no specific skin rash or lesions were identified. Her vital signs were within normal limits for temperature and respiratory rate but included severely depressed blood pressure of 65/45 mmHg and a peripheral oxygen saturation of 43%. Lung auscultation did not reveal adventitious sounds and cardiac examination was negative for murmurs or gallops. The electrocardiogram revealed ST segment elevation in leads II, III, aVF, V6 and ST segment depression in leads aVL, V1-V4 (Figure 1). Arterial Blood Gas measurements showed severe acidosis, hypokalemia, hypercapnia and hypoxia (pH: 7.08, pCO_2: 71 mmHg, pO_2: 35 mmHg, K: 2.6 mmol/L, HCO_3: 16.1 mmol/L). The patient underwent a prompt orotracheal intubation and was then transferred to the Coronary Care Unit (CCU), where she was treated with intravenous administration of normal saline, norepinephrine, dimethindene maleate, hydrocortisone sodium succinate, midazolam and ranitidine and was hemodynamically stabilized. The transthoracic echocardiography study showed

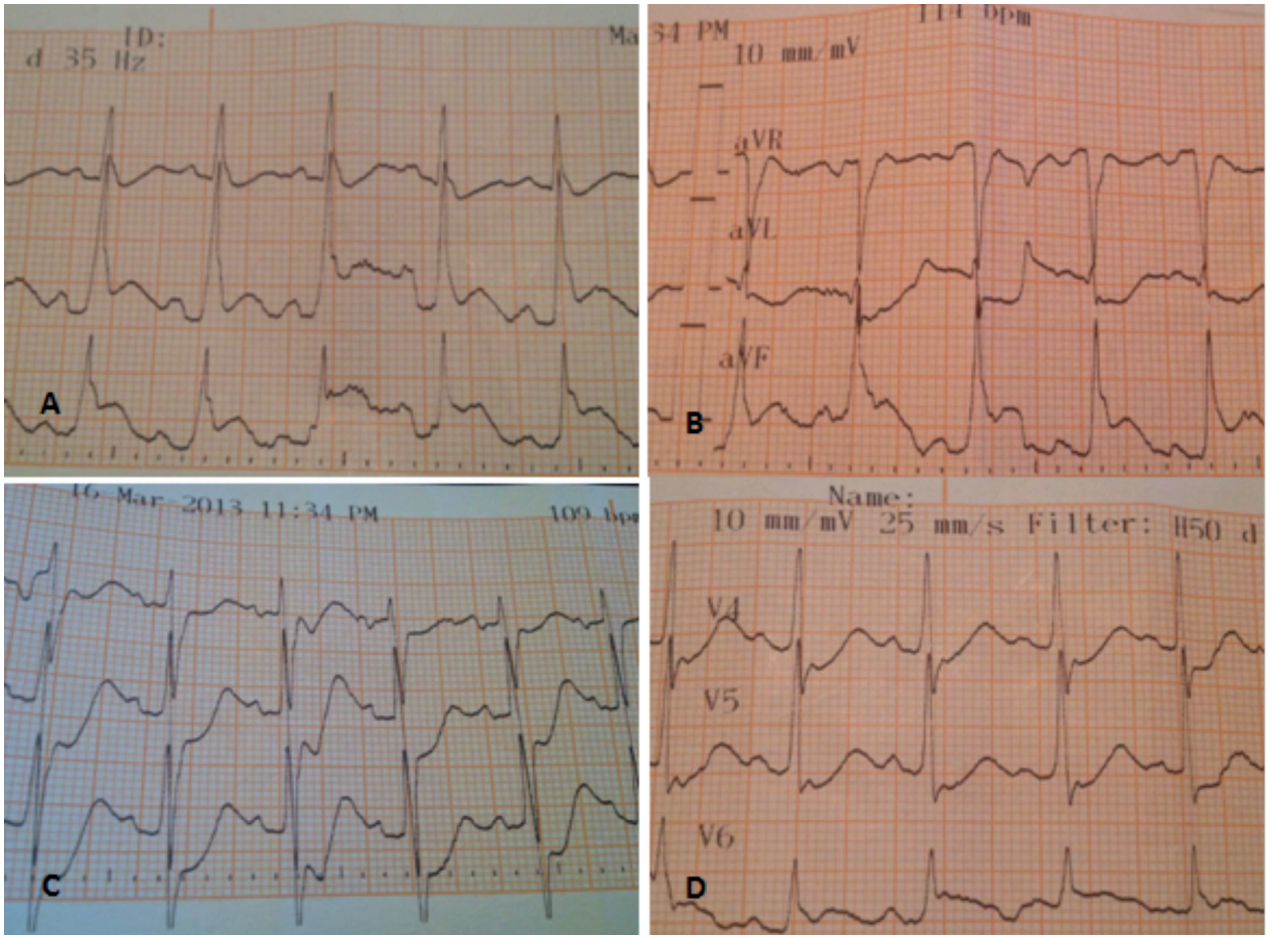

Figure 1. Admission electrocardiogram **A**. Leads I, II, III; **B**. Leads aVR, aVL, aVF; **C**. Leads V1, V2, V3; **D**. V4, V5, V6

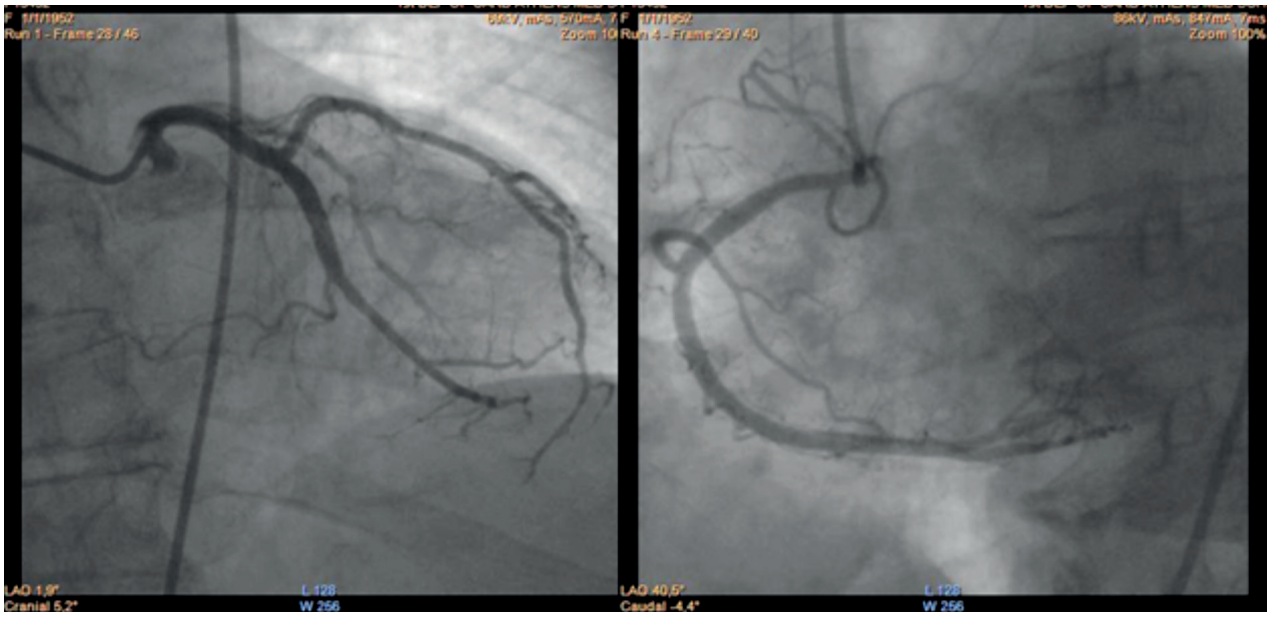

Figure 2. Coronary angiography of i. Left Descending Artery and Left Circumflex Artery in Left anterior oblique (LAO-2°) view with 5° cranial angulation; ii. Right Coronary Artery (RCA) in Left anterior oblique (LAO-40°) view with 4° caudal angulation

normal-sized cardiac chambers and normal left and right ventricular systolic function with normal valvular structure. Finally, troponin I was 0.17 ng/ml [reference values: <0.04 ng/ml], indicative of myocardial injury.

After successful weaning from inotropes (6 hours from admission to CCU, Day 1), a coronary angiogram was performed and revealed coronary arteries without significant stenoses (Figure 2). The patient returned to the CCU and was extubated the following day (Day 2), showing a sinus rhythm, and no ST-segment or T-wave abnormalities on ECG. Peak troponin I was 0.76 ng/mL and returned to normal the 5th day of hospitalization. Due to severity of anaphylaxis skin prick tests and/or oral challenge were not carried out on ethical grounds. The patient was discharged 6 days after the admission with the diagnosis of Kounis type I variant syndrome.

Discussion

The described patient developed anaphylactic reaction with shortness of breath, dizziness, sweating, pruritus, erythema, vomiting and profound hypotension with oxygen desaturation 20 minutes following the consumption of a green home-made salad that included vegetables, tomatoes and peppers. The accompanied electrocardiographic changes together with the increased of serum troponin were suggestive of inferolateral myocardial injury. This patient had suffered allergic reactions in the past and was considered as an atopic patient.

Tomatoes, green peppers and vegetables are among the most common and consumed foods worldwide. However, tomato (Solanum lycopersicum) is a common source of plant food allergens and allergic reactions, frequently occurring also in patients suffering from birch pollen allergy. Approximately 1.5% of the population in Northern Europe and up to 16% in Mediterranean basin, indeed, displays some degree of allergy towards tomato (4). The tomato allergen Sola l 4 has similar protein content with the major birch pollen allergen Bet v 1, thus making patients to cross-react easily with allergenic proteins from tomato as well as other fruits or vegetables (5).

On the other hand, the green and red pepper (Capsicum annuum) allergens osmotin, or thaumatin-like protein (Cap a 1, 23 kDa), and profilin (Cap a 2, 14 kDa) act as plant panallergens and are involved in cross-reactivity between pollen and various vegetable

foods (6). Therefore, all the ingredients of the salad consumed by the described patient, could have acted as allergens able to induce anaphylaxis and the Kounis syndrome. It is known that the more allergens an atopic patient is exposed to, the easier and quicker anaphylactic shock and Kounis syndrome occur (7). At the best of our knowledge, this is the first case of Kounis syndrome to be reported following tomato salad consumption. Kounis syndrome is defined as an acute coronary syndrome that manifests as unstable vasospastic or nonvasospastic angina, and even as acute myocardial infarction (AMI) triggered by the release of inflammatory mediators following an allergic insult (8,9). Currently, 3 variants of Kounis syndrome are identified. The first variant (Type I) includes patients with normal coronary arteries, without predisposing factors for coronary artery disease, in whom the acute release of inflammatory mediators can induce either coronary artery spasm without increase of cardiac necrosis enzymes or coronary artery spasm progressing to AMI with raised markers of myocardial injury. The second variant (Type II) includes patients with quiescent pre-existing atheromatous disease in whom the acute release of inflammatory mediators can induce either coronary artery spasm with normal cardiac enzymes or plaque erosion/ rupture manifesting as AMI. The third variant (Type III) includes patients with coronary thrombosis (including stent thrombosis) in whom aspirated thrombus specimens stained with hematoxylin-eosin and Giemsa demonstrate the presence of eosinophils and mast cells respectively (10).

Along this line, the term "cardiac anaphylaxis" refers to the functional and metabolic changes in the heart caused by the release of histamine and metabolites arising from the arachidonic acid cascade following a serious allergic insult. Several pathophysiologic mechanisms have been described to explain the involvement of this organ in anaphylactic reactions. The existence of mastocytes in heart tissue and their participation in the anaphylactic reaction that triggers tachycardia, coronary vasoconstriction, dysfunctional ventricular contractility, and blockade of atrioventricular conduction is well known (11). These abnormalities are attributed to the release of mediators such as histamine, thromboxane, prostaglandins, leukotrienes, and platelet activation factor. The release of renin during episodes of anaphylaxis and its involvement in consequent myocardial dysfunction has been described as well (12).

Several allergens have been reported to trigger Kounis syndrome, such as drugs, hymenoptera or spider venom, food, latex, and contrast media (13-15). In our case, the patient reported consumption of a green salad, which may has triggered the allergic reaction mediating the ST elevation myocardial infarction with normal coronary arteries. A sound association of the previous two episodes of allergic reaction after exposure of the patient to identical or relevant stimuli (i.e. green vegetables, tomatoes, peppers) was not established. It is known, however, that patients suffering from atopic diathesis with previous history of allergic reactions, as the described patient, are at a higher risk of hypersensitivity, allergy and anaphylaxis (14).

Conclusion

We report on a rare cause of Kounis anaphylaxis-associated acute coronary syndrome. We acknowledge that the exact pathophysiologic mechanisms of Kounis syndrome are not fully elucidated. However, Kounis syndrome should always be taken into consideration in the differential diagnosis of ischemic heart disease in patients with medical history of previous allergic reactions, who experience acute coronary syndrome after exposure to certain environmental stimuli including the commonly used tomato salad.

Conflict of interest: None to declare

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Case report

Treatment of haemorrhoidal disease with micronized purified flavonoid fraction and sucralfate ointment

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Summary. Hemorrhoidal disease is a very common disease characterized by the presence of a mucous prolapse of the rectum and by varicosis of the hemorrhoidal plexus. Medical therapy is mainly indicated for the treatment of symptoms such as bleeding, pain and itching. The use of the micronized purified flavonoid fraction (MPFF) has proven to be effective in treating symptoms of hemorrhoidal disease. Topical use of sucralfate has shown good results in the reduction of hemorrhoidal pain and itching. Our experience with three cases treated with combined use of MPFF and a topical medical device in the form of rectal ointment, composed by sucralfate and herbal (calendula, witch hazel leaf (hamamelis), chamomile) extracts, has shown good results in terms of pain and itching control and in edema reduction.

Key words: Micronized purified flavonoid fraction (MPFF), topical medical device, rectal ointment composed by sucralfate and other herbal extracts, hemorrhoidal disease, perianal pain, itching

Introduction

Haemorrhoidal disease is a very common anorectal condition defined as the symptomatic enlargement and distal displacement of the normal anal cushions (1). It is clinically characterized by painless rectal bleeding during defecation with or without prolapsing anal tissue (1-3).

In absence of rectal prolapse the medical therapy is always recommended as first step. Surgery is indicated for high-graded internal hemorrhoids, or when non-operative approaches have failed, or complications have occurred (1-4).

Constipation and abnormal bowel habits (e.g., straining, prolonged sitting, and frequent bowel movements) can play a significant role in patients with symptomatic hemorrhoids.

For these reasons dietary modification and precaution in toilet behavior represents the first approach to haemorrhoidal disease. Medical therapy plays an important role in the treatment of hemorrhoidal disease (2-5). Over the years, numerous drugs have been proposed both orally and as topical ointments.

Numerous studies have shown the efficacy of phlebotonics in the treatment of hemorrhoidal disease symptoms (2-6). The availability of a new topical medical device in the form of rectal ointment composed by sucralfate and herbal (calendula, witch hazel leaf (hamamelis), chamomile) extracts , was an opportunity to evaluate the effectiveness of the combined therapy of MPFF tablets and the topical rectal ointment in three patients who came to our attention for II-III degree hemorrhoidal disease according to Goligher classification.

Therapy

The main goal of medical treatment is to control acute symptoms of hemorrhoids rather than to cure the underlying hemorrhoids. There are several modern drugs and traditional medicine used which are available in a variety of format including pill, suppository, cream and wipes. However, the published literature lacks strong evidence supporting the true efficacy of topical treatment for symptomatic hemorrhoids.

MPFF is one of the most common oral phlebotonic drug used for treating hemorrhoids. It is apparent that flavonoids could increase vascular tone, reduce venous capacity, decrease capillary permeability, facilitate lymphatic drainage and has anti-inflammatory effects (4-6). Sucralfate is the aluminium hydroxide salt of the disaccharide sucrose octasulfate. For more than three decades, sucralfate has been used as a cytoprotective agent for treatment of gastrointestinal ulcer diseases. This salt has antimicrobial and antioxidant activity, stimulates the secretion of prostaglandin E2 (PGE2) and subsequent increased blood flow and mucus formation, and enhances the production of epidermal growth factor (EGF) which can lead to increased angiogenesis (7-8).

In the three clinical cases here reported it has been used a combo treatment of MPFF in tablets and a topical medical device in the form of rectal ointment, composed by sucralfate and herbal extracts. After three weeks, it has been evaluated the result in terms of pain, itching control, edema reduction. Moreover patient's perception of symptoms healing has been assessed with a satisfaction score from 0 to 5.

Clinical Cases

Case 1

40-year-old female who came to our attention for the presence of III degree mucohemorrhoidal prolapse. In anamnesis 2 natural births, no significant pathology. The symptomatic picture was characterized by perianal pain and rectorrhagia. Itching was also experienced. On objective examination, presence of III degree mucohemorrhoidal prolapse associated with thrombosis of the hemorrhoidal plexus at 11 o'clock.

The patient was treated for 3 weeks with oral assumption of MPFF 500 mg twice daily and topical application of rectal ointment composed by sucralfate and herbal extracts twice daily.

Pain and edema progressively decreased in the first 2 weeks and disappeared in the third week (See Figure 1). Patient perception of symptoms healing assessed with a satisfaction score from 0 to 5 showed a good perception of this patients with a score of 4.

Case 2

58-year-old female who underwent to office visit for the presence of III degree mucohemorrhoidal prolapse. In anamnesis 1 natural birth, arterial hypertension, type II diabetes and umbilical hernia. The patient had previously undergone appendectomy and cholecystectomy. She suffered from constipation, rectal bleeding and pain. Rectal exam showed a III degree mucohemorrhoidal prolapse associated to hyperemia and edema of the internal hemorrhoidal plexus. She was treated for 3 weeks with oral assumption of MPFF 500 mg twice daily and topical application of rectal ointment composed by sucralfate and herbal extracts twice daily. After 3 weeks of the combo therapy we observed a complete resolution of the hyperemia with a reduced incidence of rectal bleeding and the disappearance of pain, a significant reduction of edema and even itching was reduced (see figure 2). This patient also showed a good perception of symptoms healing with a satisfaction score of 5.

Case 3

47-year-old male who came to our attention for the presence of II-III degree mucohemorrhoidal prolapse. In anamnesis appendectomy, splenectomy and no other significant pathology. The symptoms were rectorrhagia, pain and itching. On objective examination, presence of II-III degree mucohemorrhoidal prolapse with generalized edema of the mucosa. The therapy was conducted for 3 weeks with oral assumption of MPFF 500 mg twice daily and topical application of rectal ointment composed by sucralfate and herbal

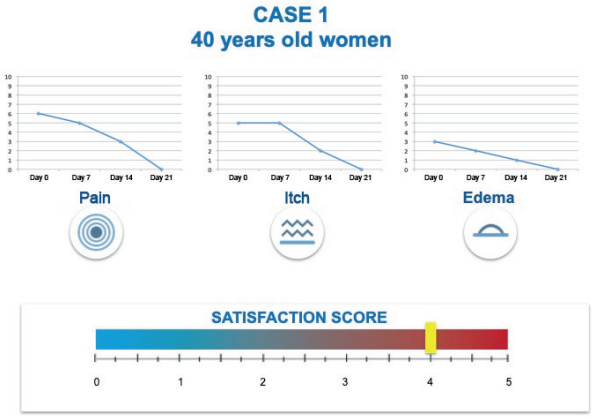

Figure 1.

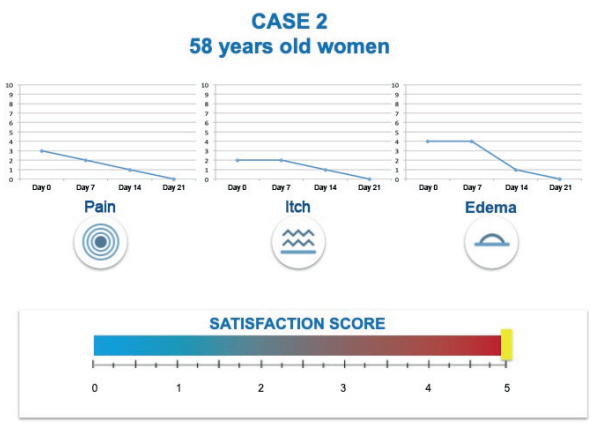

Figure 2.

extracts twice daily. After three weeks we assisted to a progressive resolution of the pain and itching and to a reduction of the edema (see Figure 3). Patient satisfaction score at the end of the combo therapy was 4.

Conclusion

Haemorrhoidal disease is the cause of most proctologic complaints and hundreds of medical and surgical therapies have been proposed to relieve symptoms. However, the role and the correct indication of medical treatments are still controversial and rarely supported by adequate trials, but in our experience the combined use of MPFF and topical application of a medical device in the form of rectal ointment composed by sucralfate and herbal extracts, seems to have a benefic effect in terms of hemorrhoidal disease symptoms control.

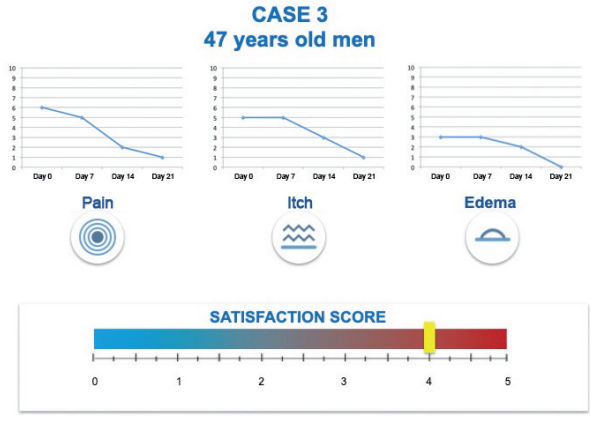

Figure 3.

Conflict of interest: Meucci and Mari declare that they have no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article. Amaturo declare that he has commercial associations with Servier Italia.

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Dasatinib induced pleuro-pericardial effusion

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To the editor:

We read with great interest the article by Abdullah et al. where they described an interesting cytological variant of chronic myeloid leukemia successfully treated with dasatinib (1). We wish to complement the article with our experience to further enhance the imparted knowledge by highlighting a rather rare complication of treatment with dasatinib, which was not emphasized in the above mentioned article for the benefit of the readers. A 61-year-old female with a past medical history of hyperlipidemia was diagnosed to have CML 4 years ago. She had been on dasatinib with complete hematologic and cytogenetic remission. She presented to ER with insidious onset, persistent, worsening shortness of breath and mild, nonproductive cough of 1 week duration. She denied any history of fever, chills, chest pain, and loss of appetite or weight. At presentation, her vitals were stable and she was saturating 98% in 2 L oxygen. Respiratory examination revealed stony dull note on percussion and absent breath sound over the right axillary, interscapular and infrascapular area. CXR confirmed a right-sided pleural effusion (Figure 1A). CT showed large, right pleural effusion with an adjacent collapse of the right lower lobe, consistent with atelectasis. It also showed pericardial effusion with thickening (Figure 1B and 1C). A diagnostic thoracentesis revealed an exudative effusion with low adenosine deaminase and negative cytology. Multiple blood, sputum, and pleural fluid culture were negative. Dasatinib induced pleuro-pericardial effusion was diagnosed and dasatinib was stopped. Following clinical improvement, she was discharged in a stable condition, with the plan of initiating an alternate tyrosine kinase inhibitor Nilotinib.

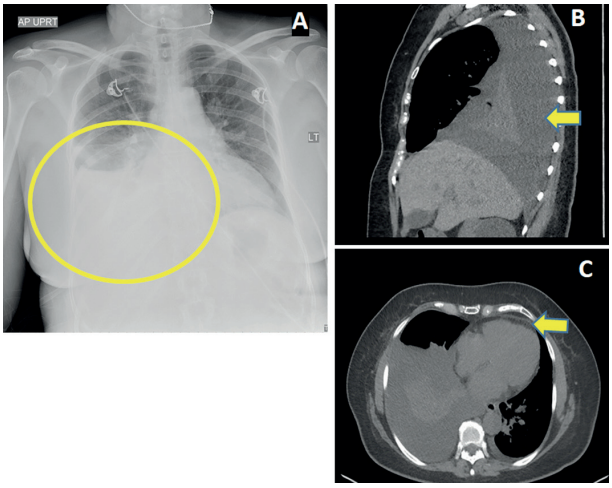

Figure 1. A: Chest radiograph showing right sided pleural effusion and enlargement of cardiac silhouette. **B:** Coronal view of computed tomography chest showing pleural effusion. **C:** Transverse view of CT chest showing both pleural and pericardial effusion

Dasatinib is a tyrosine kinase inhibitor used in chronic myeloid leukemia (CML). All tyrosine kinase inhibitors (TKI) can lead to serosal inflammation/serositis hence cause complication of pleural effusion. When used in the treatment of CML, Dasatinib can cause pleural effusion in 10 to 35% of patients (2). Off-target actions of TKIs are postulated to be the mechanism. Effusion can occur any time during treatment and commonly characterized as exudative, lymphocyte predominant effusion, of mild to moderate in severity (3). Concurrent pericardial effusion is seen in 29% of cases. Risk factors are hypertension, hyperlipidemia, previous cardiac disease and rash in response to TKI therapy (2, 3). Other cardiac complications are pulmonary artery hypertension, congestive heart failure,

pericardial effusion, QTc prolongation, and sudden cardiac death (3). Management of Dasatinib-induced pleural effusion includes dose interruptions, reductions, or permanent discontinuation. Steroids, diuretics, and therapeutic paracentesis have also been tried (3).

In conclusion among the TKIs pleuro-pericardial effusion is most commonly seen with Dasatinib. Before attributing the serositis to TKIs, infections and underlying malignancy should be ruled out (2, 4-7).

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Dasatinib induced pleural effusions - Reply

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Thanks for the reply with the title: "Dasatinib Induced Pleuro – Pericardial Effusion", we will discuss this issue in more details.

Dasatinib (Sprycel; Bristol-Myers Squibb, New York, NY) is a second-generation tyrosine-kinase inhibitor (TKI) approved for the first- and second-line treatment of chronic myeloid leukemia (CML) patients. It has been approved in the US and Europe since 2006. Dasatinib targets most imatinib-resistant BCR-ABL mutations (except the T315I and F317V mutants) by distinctly binding to active and inactive ABL-kinase. Kinase inhibition halts proliferation of leukemia cells. It also inhibits SRC family (including SRC, LKC, YES, FYN); c-KIT, EPHA2 and platelet derived growth factor receptor (PDGFRβ).

The current recommended starting doses of dasatinib are: 100 mg daily for CML in chronic phase, and 140 mg daily for CML in accelerated or blast phase. With these doses, toxicity is not uncommon, with hematologic toxicity being the most common.

It should be kept in mind that dasatinib is metabolized by CYP3A4 system, so if administered concomitantly with strong CYP3A4 inhibitors and grapefruit juice, toxicity will increase, so the dose should be reduced. Conversely, if administered concomitantly with strong CYP3A4 inducers and St John's wort, the dose should be increased with close monitoring.

Of the Bcr-Abl TKIs, dasatinib has been associated with the highest frequency of pulmonary side effects. During treatment with dasatinib, pleural, pulmonary vascular, and lung parenchymal abnormalities can develop separately or simultaneously. Between 10 and 35 percent of patients treated with dasatinib in clinical trials developed pleural effusions, most often exudative and lymphocyte predominant (1-3). Dasatinib-induced effusions may be a result of PDGFR inhibition, but some clinicians suspect that it is a result of lymphatic drainage abnormalities and microvasculopathy associated with a protein leak (2).

Optimal treatment of dasatinib-related pleural effusions, when they occur, is not known. In case series, treatment has included systemic glucocorticoids, diuretics, thoracentesis, and dasatinib interruption or discontinuation (1, 3). Rarely, pleurodesis has been used (4). Combinations of the above therapies have also been employed.

It has been shown in small studies that lower doses of dasatinib are better tolerated and associated with less side effects, while efficacy is not affected [100 mg daily vs higher dose (5), 50 mg daily vs higher dose (6)]. We applied this in the case we previously reported with excellent results (7).

In conclusion, pleural effusions occur in up to one-third of patients treated with dasatinib at the current recommended doses, with hematologic toxicity being much more common, so maybe in the future, with larger studies, recommended doses might be reduced.

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Ciliated nasal epithelial cells damage and human rhinovirus infection: cytological findings

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To the Editor:

We read with great interest the article by Gelardi et al. regarding ciliocytophthoria (CCT) of nasal cells in 20 patients infected with type A influenza virus, demonstrating three distinct characteristic phases of CCT (1). In another article by the same authors, 12 patients with serologically confirmed type A influenza infection of the upper airways had morphological alterations to the nuclei of their nasal cells in nasal scrapings, when stained with the May-Grünwald Giemsa preparation (2). The nuclear changes consisted primarily of coalescence of chromatin, forming a compact mass surrounded by a peripheral halo.

Viruses such as adenovirus, influenza virus and respiratory syncytial virus, are known to cause cellular damage and morphological alterations: the cytophatic effect (3).

Although in vitro and in vivo studies have shown that human rhinoviruses disrupts tight junctions complexes, with consequent loss of epithelial barrier integrity and extrusion of ciliated nasal epithelial cells (4), human rhinovirus infection has no far not been shown to demonstrate similar cellular damage (5).

Rhinovirus, coronavirus, adenovirus, respiratory syncytial virus, parainfluenza virus type 1, 2 and 3, and influenza virus type A and B can all infect ciliated nasal epithelial cells and cause the common cold, but human rhinovirus infection has been identified as the commonest cause (52.5% of cases) and is therefore particularly important from a population perspective (6). We were therefore interested in cytological assessment of nasal cells from patients with human rhinovirus infection.

Cytological examination was undertaken of nasal smears from a group of 7 patients, 4 females and 3 males; aged between 27 and 14 years-old (average = 17.5), who had longstanding perennial rhinitis (in all cases 7 years or more). They had all been treated with oral H1 antihistamines and nasal corticosteroid sprays during the preceding two years. Two patients, (27 and 21 years-old male respectively) also had nasal polyposis, and one (18 years-old female) had bronchial asthma. None of the patients were smokers. They presented at our research unit showing clinical symptoms of a common cold, including sore throat, nasal congestion, sneezing, and watery rhinorrhea. Serology and DNA real-time PCR demonstrated human rhinoviruses type A (5 cases) and C (two cases) as the cause of the infection. Nasopharyngeal swabs were taken, fixed with 96% ethanol and stained using the Papanicolaou method and Giemsa stain. The research was undertaken with full consent, and in line with obligations of research practice as outlined in the Declaration of Helsinki.

Under light microscopy the Pap smears showed the presence of numerous detached ciliated nasal epithelial cells, with approximately one third of the cells showed destruction and irregular attachment of cilia, microvacuolated cytoplasm, and large red round intranuclear bodies, similar to inclusions, surrounded by clear halos, where the nuclear borders were visible (Figure 1A). Eosinophils were very scarce (MGG

stain). Ultrastructural examination of the cells indicated disappearance of cilia, or shortened, fragmented and disordered cilia. Large intranuclear bodies appeared as a compact mass similar, similar to the condensed chromatin, and separated from the nuclear membrane by a clear space (Figure 1B). No viral particles were observed in the cytoplasm or nucleus.

Our results confirm that human rhinovirus can alter the morphology of the ciliated nasal epithelial cells, principally causing alterations to the cilia and provoking nuclear changes.

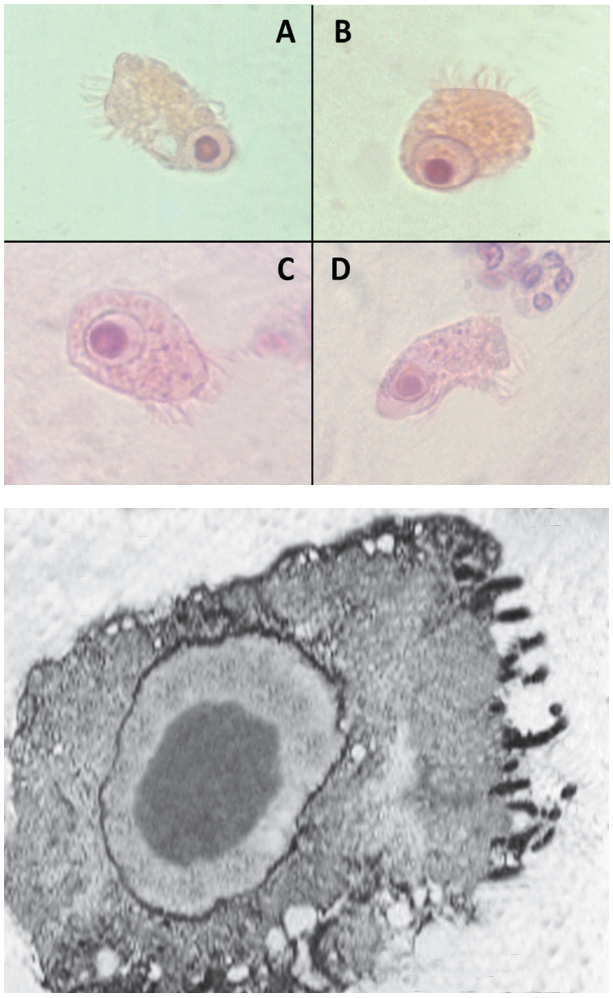

Figure 1. A: Cytophatic effect showing destruction and irregular attachment of cilia, microvacuolated cytoplasm, and large red round intranuclear bodies, surrounded by clear halos (Papanicolaou stain x 400). B: Ultrastructural findings of an infected ciliated nasal epithelial cell.

Although we had a small sample size (n=7), we think that the results are of interest. A larger sample, assessing any interplay with demographic factors such as gender, age, smoking, and presence or absence of other pathological conditions would be helpful in confirming and expanding on these findings.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Nasal cytology identifies healthy and damaged nasal epithelial cells - Reply

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To the Editor,

we would like to thank Rafael Martínez-Girón, Hugo Cornelis van Woerden, and Cristina Martínez-Torre, who confirmed post-viral, namely during rhinovirus infection, cytomorphological alterations loaded to nasal epithelial cells (1). The outcomes of that study were consistent with our research that described structural modification during viral infection (2,3). In this regard, the first evidence concerned vacuolar degeneration at the cytoplasmic level and further nuclear impairment, manly nuclear alterations, such as intranuclear halo (4). In particular, it has to note that the "halo" is inside the cellular membrane as confirmed by electronic microscopy (3).

Analyzing the subjects investigated by the authors, it seems that some relevant details were lacking, mainly concerning the type of perennial rhinitis. In fact, as reported, all subjects were treated with oral H1 antagonists and intranasal corticosteroids. Both medications are commonly prescribed for allergic rhinitis. Besides, 2 patients had nasal polyps and one asthma. There is a reasonable suspicion that they suffered from allergic rhinitis. In conflict with this hypothesis, eosinophils were, however, very scarce. Remarkably, perennial rhinitis was not classified in their study. Consistently, we would underline the clinical relevance of nasal cytology in the workup of nasal disorders (5). Nasal cytology is a simple, easy, and repeatable technique that is very fruitful in clinical practice. Nasal cytology carefully defines the phenotype and endotype of rhinitis, so it is a classic example of Precision Medicine (6) and it is a point-of-care test (7). Moreover, it has been recently standardized, thus the methodology has been rigorously validated (8). In the context of the topic, a close link exists between allergic rhinitis and rhinovirus. It was demonstrated that allergic patients have a mucosal inflammation that involves adhesion molecule machinery, mainly intercellular adhesion molecule 1 (ICAM-1), and is associated with functional impairment, such as nasal airflow limitation (9-11). Interestingly, ICAM-1 is also the main receptor for rhinovirus: this curious coincidence explains the increased susceptibility to infections in allergic patients (12). These concepts underline the importance of a precise and documented diagnosis of rhinitis that is the requisite for a tailored treatment: the so-called Personalized Medicine (13).

In conclusion, nasal cytology could be envisaged as a mandatory test to identify the phenotype, and endotype to optimize the management of patients with perennial rhinitis.

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Pros and Cons in General Medicine and Geriatrics, 2019

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The article takes into consideration a group of papers of 2019, focused on improving clinicians' abilities to treat adult and older patients, and looking at top stories physicians were talking about on media.

At the top of the list are guidelines saying that daily **low-dose aspirin** should be reserved for people with the highest cardiovascular risk and the lowest risk for bleeding. A useful meta-analysis showed that aspirin use for prevention did not reduce the risks for death, cardiovascular death, or fatal MI, while the benefits in risks for nonfatal MI and stroke seemed to be offset by bleeding risks. Estimates suggest that the risks outweigh the benefits for most primary prevention patients (1, 2). A meta-analysis offers clinicians more evidence on the benefits and harms of aspirin for the primary prevention of cardiovascular (CV) disease. Researchers examined 13 studies in which nearly 165.000 adults without CV disease were randomized to receive daily aspirin or no aspirin. Overall, during a median 5 years' follow-up, aspirin was associated with a lower risk for major CV events and a higher risk for major bleeding. The researchers estimated that 265 patients would need to be treated to prevent one CV event, and 210 to cause one bleeding event (3).

Not Quite Time for App-Detected **Atrial Fibrillation (AF).** The Apple Watch has an optical sensor that can detect heart rates.The industry-sponsored, prospective, open-label, siteless, pragmatic Heart Study tested an algorithm to identify AF; it is groundbreaking because of its massive size and siteless method. However it has many limitations: large percentages of patients were lost to follow-up and the confirmation method's sensitivity is unknown.Overall, the algorithm caught some AF episodes and missed others. At the moment we should not rely on the Apple Watch, but neither can we ignore this new opportunity. We certainly need more study about how to optimize such tools and, especially, how best to respond to brief, intermittent, subclinical AF episodes (4).

A**blating Atrial Fibrillation** (AF) in Heart Failure Patients: AF frequently accompanies heart failure, particularly with reduced ejection fraction (HFrEF). A rhythm control strategy theoretically offers the potential for clinical benefit, but antiarrhythmic agents have not been proven to improve outcomes. Catheter ablation of AF might provide clinical benefit without the toxicities of antiarrhythmic agents but might involve procedural risks. The current researchers conducted a meta-analysis of six randomized, controlled trials, including CASTLE-AF, of AF ablation in patients with HFrEF. In the 775 patients, ablation compared with physician-directed medication (including rate-controlling agents or antiarrhythmic agents) was associated with lower rates of all-cause mortality (9.0% vs. 17.6%) and HF hospitalizations (16.4% vs. 27.6%). The two approaches had no significant differences in adverse events. AF ablation was also beneficial in improving left ventricular ejection fraction, 6-minute walk distances, and quality of life, and it could be extremely of benefit in older subjects.(5)

What the available data to tell us about how many older people are taking **statins** for primary prevention, and how much good is it likely doing them? The role of statin therapy in primary prevention of cardiovascular

disease in persons older than 75 years remains a subject of debate. An analysis of guideline-driven indications for statin use concludes that the drugs, especially when used for primary prevention in low-risk patients, may constitute low-value care. Researchers writing in The BMJ examined changes in eligibility for guideline-directed statin use between 1987 and 2016. They found that the proportion of people older than 50 in Ireland eligible for statin treatment rose from 8% to 61% over that period. As a result, the number needed to treat with statins to prevent one major cardiovascular event rose tenfold, from 40 to 400. Although there are clear benefits for high risk groups, the authors remind readers that statins' net benefits depend on the individual patient's baseline risks. For example, age, smoking status, and cholesterol status. With wide confidence intervals around measures of relative risk among patients at low baseline risk, the drug's effects also encompass the possibility of harm. This analysis should prompt physicians to think carefully about how they recommend statins for primary prevention (6). In spite of this, a nationwide study of 120.173 people in France, who were aged 75 between 2012 and 2014 and had been taking statins continuously for two years, has found those who stopped taking their statins had a 33% increased risk of being admitted to hospital with heart or blood vessel problems during an average follow-up period of 2.4 years. The study is the first to evaluate the impact of discontinuing statins taken for primary prevention in older people. The researchers stress that this is an observational, retrospective, non-randomised study and therefore cannot show that discontinuing statins can cause a heart attack or stroke, only that it is associated with it. Limitations of the study include the fact that statin use was defined by prescriptions dispensed, although the researchers point out that as the patients regularly had prescriptions dispensed to them, they would be unlikely not to take the medication; the researchers did not have information on patients' socioeconomic status, their lifestyles, cholesterol levels at the start of the period being studied, tobacco use, obesity and frailty; and they did not have precise information on the reasons why people stopped taking statins (7).

Concerns regarding statin use and accelerated cognitive decline and/or memory loss keep surfacing in mainstream media. These messages generated confusion as well as hurting statin adherence. But a recent paper concluded that the outcomes of the analysis of many researches were reassuring and confirmed earlier findings that refuted the suggested link between statin use and cognitive decline (8).

Another very important problem in older patients are Statin Associated Muscolar Simptoms, with the increased risk of falling and reduction of gait speed and motility . It is up to the clinician to decide to stop or to continue statin, taking into consideration risk and benefits and willings of any patient.

Ultrasound Screening for Abdominal Aortic Aneurysms: The USPSTF reviewed the most recent evidence on screening for abdominal aortic aneurysms (AAAs; defined as aortic enlargement of ≥3.0 cm in diameter). One-time screening of older men (age range 65-75) who have ever smoked (at least 100 lifetime cigarettes) confers benefit of moderate certainty (B recommendation). Benefit of screening in older men who have never smoked is less clear and should be offered selectively, based on family history and risk factors such as history of other aneurysms, coronary or cerebrovascular disease, hyperlipidemia, and hypertension (C recommendation). Screening older women who have never smoked and have no family history of AAA likely will cause more harm than benefit (D recommendation). The Task Force found insufficient evidence to make a recommendation about screening older women who have ever smoked or who have family history of AAA. Maybe that these recommendations are influenced by the improved outcomes with endovascular repair that now is used preferentially over open repair. Optimal screening intervals for patients with aneurysms smaller than 5.5 cm in diameter are unknown (9-11).

Diabetes Groups Refine Recommendations for **Glucose-Lowering Drugs**. In certain high-risk people with diabetes, clinicians' decision to use a glucagon-like peptide 1 (GLP-1) receptor agonist or sodium-glucose cotransporter 2 (SGLT2) inhibitor to reduce cardiovascular and renal risk should be made without regard to baseline HbA1c or HbA1c target.

For patients with established atherosclerotic cardiovascular disease and diabetes for whom major adverse cardiovascular events are the largest threat, GLP-1 receptor agonists have the greatest benefit against CV events. This class can also be considered for patients with diabetes who don't have established CVD but are at high risk. For patients with heart failure with reduced ejection fraction or chronic kidney disease (CKD), SGLT2 inhibitors are the most beneficial option. For patients with diabetes and CKD, this class is recommended to prevent progression of CKD, heart failure hospitalization, major adverse cardiovascular events, and cardiovascular death. SGLT2 inhibitors are also recommended for people with foot ulcers and those at risk for foot amputation, but only after shared decision making about risks and benefits . In the "real world", SGLT2 use is associated with lower risks for heart failure, all-cause death, major adverse CV events, and CV-related death, findings that are consistent with those from randomized trials. Notably, the American Diabetes Association recommends an SGLT2 inhibitor or a glucagon-like peptide-1 receptor agonist as the first choice for a second-line agent in type 2 diabetics with CV disease, heart failure, or chronic kidney disease (12).

Acupuncture may help ease pain in patients with cancer? A meta-analysis compared acupuncture or acupressure with control therapies (placebo, sham acupuncture, analgesics, usual care) in over 900 cancer patients. Compared with sham acupuncture, real acupuncture was associated with reduced cancer pain intensity. Pain intensity was also lower for patients who received acupuncture or acupressure plus analgesics, relative to those who received only analgesics. Two studies found that patients assigned to acupuncture used lower analgesic doses than those who used only analgesics. We need more rigorous trials to identify the association of acupuncture and acupressure with specific types of cancer pain and to integrate such evidence into clinical care to reduce opioid use (13).

Measuring NT-ProBNP for **Preoperative Cardiac Risk Assessment?** Postoperatively, troponin T levels were measured daily (for as long as 3 days) to detect myocardial injury after noncardiac surgery (MINS). Patients were stratified into four risk groups by preoperative NT-proBNP levels: <100, 100-199, 200-1499, and ≥1500 pg/mL. The overall incidence of MINS was 12%; most cases were asymptomatic elevations in troponin (and not clinical myocardial infarctions). In adjusted analyses, patients with increasingly higher NT-proBNP levels in the four risk groups had progressively – and significantly – higher incidences of MINS (5%, 12%, 21%, and 38%), vascular-related death (0.2%, 0.4%, 0.7%, and 2.9%), and all-cause mortality (0.3%, 0.7%, 1.4%, and 4.0%) within 30 days after surgery. In middle-aged or older adults (age, ≥45) who are scheduled for inpatient noncardiac surgery, adding NT-proBNP to the RCRI score could improve individualized preoperative risk stratification and could help patients decide whether potential risks associated with elective noncardiac surgery are acceptable. In theory, preoperative NT-proBNP levels also could help clinicians adjust preoperative investigations, guide surgical or anesthetic approaches, and more precisely intensify or lessen postoperative monitoring. However, whether additional diagnostic and therapeutic interventions triggered by routine perioperative measurement of NT-proBNP would lower postoperative morbidity and mortality is unclear (14).

Managing **Dyspepsia** with "Test and Treat" ranked best strategy (15). Five management strategies were compared: 1- Prompt endoscopy 2-Test for H. pylori, followed by endoscopy after positive findings 3-"Test and treat" (for the presence of H. pylori) 4-Empirical acid suppression 5- Symptom-based management .The approach resulting in the lowest risk of remaining symptomatic by the trials' final follow-up was "test and treat," with a relative risk of 0.89. That strategy just beat out prompt endoscopy (RR, 0.90). However, no strategy was clearly superior to any other. Prompt endoscopy gained the highest patient-satisfaction ratings; however, the authors point to the high costs involved with that approach. This is a very practical analysis of treatment modalities for a common problem in primary care. While no strategy was clearly superior, this study validates an inexpensive and commonly used approach by primary care clinicians that also will help patients avoid endoscopy. One of the most important findings of this study is the fact that upper gastrointestinal can-

cer rates were very low in all trials. This should give us comfort that each of these strategies is reasonable, with very little risk of adverse patient outcomes.

Over 40% of **Antibiotic Prescriptions** potentially inappropriate. Researchers studied antibiotic prescribing practices in a nationally representative sample of 28.000 ambulatory care visits from 2015. Among the other findings: roughly 25% of antibiotic prescriptions were inappropriate (e.g., for upper respiratory tract infection), and 18% lacked a documented indication. Primary care clinicians had lower rates of prescriptions without a documented indication (12%) than other specialists who often prescribe antibiotics (24%) or all other specialists (29%). When a culture was taken, clinicians were less likely to prescribe without an indication. Prescriptions for sulfonamides and urinary antiinfectives had higher rates of no documented indication than penicillins. Nearly one in five antibiotic prescriptions lacked documented indications. Some, if not many, of prescriptions might have been appropriate (e.g., a clinician reasonably might suspect a urinary tract infection and prescribe an anti-infective agent but code for a nonspecific symptom such as dysuria). Of course, inappropriate antibiotic prescribing should be avoided altogether (16).

Testosterone therapy can induce hematologic abnormalities associated with hypercoagulability, but whether it actually confers excess risk for venous thromboembolism (VTE) is controversial. When use of testosterone during the 6 months immediately preceding VTE (case period) was compared with use of testosterone during months 6 to 12 prior to VTE (the control period), testosterone therapy was associated significantly with development of VTE (odds ratio, ≈2.0). Outcomes were similar in patients with or without coded diagnoses of hypogonadism. Medical claims and pharmacy data have many potential sources of error, including inaccuracy in capturing all hypogonadism diagnoses. This study emphasizes the proximate nature of testosterone therapy and incident VTE, not necessarily the absolute risk for VTE with or without testosterone therapy (17). The American College of Physicians suggests that clinicians discuss whether to start testosterone therapy with men who have age-related low testosterone and want to treat their sexual dysfunction, according to new guidelines published in the Annals of Internal Medicine (18). Testosterone is not recommended for other nonspecific symptoms of aging, like fatigue or cognitive decline. For men treated with testosterone, clinicians should schedule follow-up for 12 months later and occasionally thereafter. Treatment should be discontinued in men with no improvement in sexual function. Clinicians should consider intramuscular injection over transdermal application because of lower costs (18).

What is the best first-line treatment for patients with **hypertension**? According to current guidelines, based largely on randomized trials that are more than 2 decades old, thiazide or thiazide-like diuretics, angiotensin-converting-enzyme (ACE) inhibitors, angiotensin-receptor blockers (ARBs), and calcium-channel blockers (CCBs) are acceptable first-line therapies Researchers analyzed data from nine large observational databases from four countries (with data on 4.9 million patients with as long as 22 years of follow-up) and synthesized tens of thousands of between-drug comparisons in patients whose initial treatment was a single drug from any of the aforementioned classes (median patient follow-up, ≈2 years). Multiple advanced statistical techniques were applied to minimize confounding. ACE inhibitors were used most often for initial monotherapy (48% of patients), followed by thiazide or thiazide-like diuretics (17%), dihydropyridine calcium-channel blockers (e.g., amlodipine; 16%), angiotensin-receptor blockers (15%), and non-dihydropyridine calcium-channel blockers (e.g., diltiazem; 3%). Most comparisons showed no significant difference in incidence of the combined primary outcome (i.e., myocardial infarction, hospitalization for heart failure, and stroke) between classes, but thiazides conferred a significantly lower risk for all three outcomes relative to ACE inhibitors (hazard ratios, ≈0.8 for all outcomes), and non-dihydropyridine CCBs significantly underperformed all other classes. Compared with ACE inhibitors, thiazides were associated with significantly lower rates for 16 of 46 adverse safety outcomes. This massive data analysis, although retrospective, reveals real-world outcomes on a scale far beyond any conceivable randomized trial and sug-

gests that the most commonly prescribed drugs for initial antihypertensive treatment might not be the safest and most effective. In patients beginning hypertension monotherapy, diuretics are a more effective and safer option than angiotensin-converting enzyme (ACE) inhibitors. However, it's possible that patterns of effectiveness and adverse effects across drug classes would change with longer duration of follow-up (19). Clearly the choice of antihypertensive agent depends on multiple patient-related factors such as comorbidities. Nonetheless, it is reassuring to know that diuretics, which are inexpensive and well tolerated, can be safely used as first-line treatment for hypertension (19).

Acute gout and pseudogout usually are managed with colchicine, nonsteroidal anti-inflammatory drugs, steroids, or joint injections. However, in patients with comorbid conditions such as chronic kidney disease, heart failure, diabetes, or hypertension, these standard therapies often are avoided. The interleukin 1-receptor antagonist anakinra sometimes is used off-label in patients with acute crystal disease who cannot be managed with traditional therapy, although access is restricted because of its expense. In this retrospective observational U.S. study of 100 medically complex hospitalized patients (mean age, 60) with acute gout or pseudogout, researchers evaluated anakinra's efficacy and safety. Previous case reports of anakinra use for acute gout and pseudogout have been published, but this is the largest observational study in an inpatient setting; 75% of patients significantly improved within 4 days. No important adverse events occurred, even among these complex patients. This study, although retrospective and uncontrolled, offers support for anakinra treatment of acute crystal disease in patients with substantial comorbidities (20).

In older patients, **polypharmacy** (5 or more regular prescriptions) is associated with risks for falls, disability, and death, over and above risks associated with the illnesses the drugs are intended to treat. To examine the association between polypharmacy and falls in more detail, Canadian researchers prospectively evaluated gait patterns among 249 older adults (age ≥65) without gait-impairing neurological diagnoses. At baseline, the 176 patients who were taking more than five medications (mean, 9 prescriptions), had more diagnosed illnesses, reported more falls in the previous year, and (on formal gait testing) walked considerably more slowly and haltingly than the other 73 patients. During 5 years of follow-up, gait parameters worsened more rapidly in the high-med group, even after controlling for age and comorbidity. The researchers calculated that every additional medication increased independent risk for gait deterioration by about 15% and increased fall risk by 5%. Most patients and caregivers agreed they would be willing to stop a medication on a physician's recommendation. Both clinicians and their older patients are to blame for those long lists of pills – physicians might prefer not to tamper with stable regimens, whereas patients often become oddly attached to familiar regimens. The goal: cutting back medications to the minimum is vital, especially in frail oldest persons (21).

Nutritional Recommendations implicate unprocessed red meat and processed meat in conferring adverse cardiovascular (CV) and cancer outcomes. However, all have one or more limitations: a) they include only observational studies, with high risk for confounding; b) they lack reporting of absolute magnitude of effects; c) they lack systematic review of the evidence; d) they omit authors' conflicts of interest; and e) they inconsistently incorporate population values and preferences. An independent panel addressed each of these limitations, using findings from five comprehensive meta-analyses.The panel's evaluation showed no significant difference between subjects who consumed higher versus lower quantities of red meat during longer than 10 years of follow-up for the outcomes of all-cause mortality, CV-related mortality, CV disease, or cancer-related mortality, including colorectal cancer (low- to very low-certainty evidence). The observational studies showed that, for every 100 people who reduced processed or unprocessed meat intake by 3 servings per week, roughly 1 person avoided death and 1 person avoided a diagnosis of diabetes during 11 years of follow-up. Adults may continue moderate consumption of unprocessed red meat and processed meat (weak recommendation, based on low-certainty evidence). Greater reductions in meat consumption than the "practical" 3 servings-per-week reductions

might yield greater reductions in adverse outcomes. The slightly better outcomes seen in the observational data require individuals to maintain relevant dietary changes over very long periods (>10 years) to gain benefit; very motivated individuals who maintain large reductions (>3 servings per week) in their very long-term meat intake might glean benefits. For people who are interested in reducing meat consumption (whether to improve health or mitigate the effects of meat production on the environment), certainly nothing argues against that lifestyle modification (22-24).

Too many PROS&CONS in 2019? Let's go to **conclusion**: Three Easy Ways to Save Almost 100 Million Lives Worldwide. Noncommunicable diseases (NCDs) are the leading cause of death worldwide and are largely preventable through changes in modifiable risk factors: increasing the coverage of hypertension treatment to 70%, reducing dietary sodium by 30%, and eliminating trans fats.(25) The three interventions could delay 94.3 million deaths during 25 years (39.4 million from boosting hypertension treatment, 40.0 million from reducing sodium intake, and 14.8 million from eliminating trans fats). The effects are greater for men than women and for older (age ≥70) than younger people. Three simple population-level interventions could dramatically decrease the global burden of NCDs. To achieve this goal, low- and middle-income countries in particular need public health resources and infrastructure to increase access to pharmacotherapy for hypertension, the leading modifiable risk factor for early cardiovascular disease. The interventions are not only feasible but extremely cost-effective. The lowest-hanging fruit might be eliminating trans fats through national legislation, as has been done in some high-income countries. What is clear from this and other similar analyses is that to substantially move the needle in cardiovascular disease prevention, interventions must move beyond the level of individual patients (25).

Conflict of interest: The author declares that he has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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WHO Declares COVID-19 a Pandemic

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The World Health Organization (WHO) on March 11, 2020, has declared the novel coronavirus (COVID-19) outbreak a global pandemic (1).

At a news briefing , WHO Director-General, Dr. Tedros Adhanom Ghebreyesus, noted that over the past 2 weeks, the number of cases outside China increased 13-fold and the number of countries with cases increased threefold. Further increases are expected. He said that the WHO is "deeply concerned both by the alarming levels of spread and severity and by the alarming levels of inaction," and he called on countries to take action now to contain the virus. "We should double down," he said. "We should be more aggressive."

Among the WHO's current recommendations, people with mild respiratory symptoms should be encouraged to isolate themselves, and social distancing is emphasized and these recommendations apply even to countries with no reported cases (2).

Separately, in JAMA, researchers report that SARS-CoV-2, the virus that causes COVID-19, was most often detected in respiratory samples from patients in China. However, live virus was also found in feces. They conclude: "Transmission of the virus by respiratory and extrarespiratory routes may help explain the rapid spread of disease."(3).

COVID-19 is a novel disease with an incompletely described clinical course, especially for children. In a recente report W. Liu et al described that the virus causing Covid-19 was detected early in the epidemic in 6 (1.6%) out of 366 children (≤16 years of age) hospitalized because of respiratory infections at Tongji Hospital, around Wuhan. All these six children had previously been completely healthy and their clinical characteristics at admission included high fever (>39°C) cough and vomiting (only in four). Four of the six patients had pneumonia, and only one required intensive care. All patients were treated with antiviral agents, antibiotic agents, and supportive therapies, and recovered after a median 7.5 days of hospitalization. (4).

Risk factors for severe illness remain uncertain (although older age and comorbidity have emerged as likely important factors), the safety of supportive care strategies such as oxygen by high-flow nasal cannula and noninvasive ventilation are unclear, and the risk of mortality, even among critically ill patients, is uncertain. There are no proven effective specific treatment strategies, and the risk-benefit ratio for commonly used treatments such as corticosteroids is unclear (3,5).

Septic shock and specific organ dysfunction such as acute kidney injury appear to occur in a significant proportion of patients with COVID-19–related critical illness and are associated with increasing mortality, with management recommendations following available evidence-based guidelines (3).

Novel COVID-19 "can often present as a common cold-like illness," wrote Roman Wöelfel et al. (6). They report data from a study concerning nine young- to middle-aged adults in Germany who developed COVID-19 after close contact with a known case. All had generally mild clinical courses; seven had upper respiratory tract disease, and two had limited involvement of the lower respiratory tract. Pharyngeal virus shedding was high during the first week of symptoms, peaking on day 4. Additionally, sputum viral shedding persisted after symptom resolution. The German researchers say the current case definition for COVID-19, which emphasizes lower respiratory tract disease, may need to be adjusted(6). But they considered only young and "normal" subjecta where-

as the story is different in frail comorbid older patients, in whom COVID 19 may precipitate an insterstitial pneumonia, with severe respiratory failure and death (3).

High level of attention should be paid to comorbidities in the treatment of COVID-19. In the literature, COVID-19 is characterised by the symptoms of viral pneumonia such as fever, fatigue, dry cough, and lymphopenia. Many of the older patients who become severely ill have evidence of underlying illness such as cardiovascular disease, liver disease, kidney disease, or malignant tumours. These patients often die of their original comorbidities. They die "with COVID", but were extremely frail and we therefore need to accurately evaluate all original comorbidities.

In addition to the risk of group transmission of an infectious disease, we should pay full attention to the treatment of the original comorbidities of the individual while treating pneumonia, especially in older patients with serious comorbid conditions and polipharmacy. Not only capable of causing pneumonia, COVID-19 may also cause damage to other organs such as the heart, the liver, and the kidneys, as well as to organ systems such as the blood and the immune system. Patients die of multiple organ failure, shock, acute respiratory distress syndrome, heart failure, arrhythmias, and renal failure (5,6).

What we know about COVID 19?

In December 2019, a cluster of severe pneumonia cases of unknown cause was reported in Wuhan, Hubei province, China. The initial cluster was epidemiologically linked to a seafood wholesale market in Wuhan, although many of the initial 41 cases were later reported to have no known exposure to the market (7).

A novel strain of coronavirus belonging to the same family of viruses that cause severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), as well as the 4 human coronaviruses associated with the common cold, was subsequently isolated from lower respiratory tract samples of 4 cases on 7 January 2020 .

On 30 January 2020, the WHO declared that the SARS-CoV-2 outbreak constituted a Public Health Emergency of International Concern, and more than 80, 000 confirmed cases had been reported worldwide as of 28 February 2020 (8). On 31 January 2020, the U.S. Centers for Disease Control and Prevention announced that all citizens returning from Hubei province, China, would be subject to mandatory quarantine for up to 14 days . But from China COVID 19 arrived to many other countries. Rothe C et al reported a case of a 33-year-old otherwise healthy German businessman :she became ill with a sore throat, chills, and myalgias on January 24, 2020 (9). The following day, a fever of 39.1°C developed, along with a productive cough. By the evening of the next day, he started feeling better and went back to work on January 27.Before the onset of symptoms, he had attended meetings with a Chinese business partner at his company near Munich on January 20 and 21. The business partner, a Shanghai resident, had visited Germany between January 19 and 22. During her stay, she had been well with no signs or symptoms of infection but had become ill on her flight back to China, where she tested positive for 2019-nCoV on January 26.

This case of 2019-nCoV infection was diagnosed in Germany and transmitted outside Asia. However, it is notable that the infection appears to have been transmitted during the incubation period of the index patient, in whom the illness was brief and nonspecific.The fact that asymptomatic persons are potential sources of 2019-nCoV infection may warrant a reassessment of transmission dynamics of the current outbreak (9).

Our current understanding of the incubation period for COVID-19 is limited. An early analysis based on 88 confirmed cases in Chinese provinces outside Wuhan, using data on known travel to and from Wuhan to estimate the exposure interval, indicated a mean incubation period of 6.4 days (95% CI, 5.6 to 7.7 days), with a range of 2.1 to 11.1 days. Another analysis based on 158 confirmed cases outside Wuhan estimated a median incubation period of 5.0 days (CI, 4.4 to 5.6 days), with a range of 2 to 14 days . These estimates are generally consistent with estimates from 10 confirmed cases in China (mean incubation period, 5.2 days [CI, 4.1 to 7.0 days] and from clinical reports of a familial cluster of COVID-19 in which symptom onset occurred 3 to 6 days after assumed exposure in Wuhan (10-12).

The incubation period can inform several important public health activities for infectious diseases,

including active monitoring, surveillance, control, and modeling. Active monitoring requires potentially exposed persons to contact local health authorities to report their health status every day. Understanding the length of active monitoring needed to limit the risk for missing infections is necessary for health departments to effectively use resources. A recent paper provides additional evidence for a median incubation period for COVID-19 of approximately 5 days (13). Lauer et al suggest that 101 out of every 10 000 cases will develop symptoms after 14 days of active monitoring or quarantinen (13) . Whether this rate is acceptable depends on the expected risk for infection in the population being monitored and considered judgment about the cost of missing cases. Combining these judgments with the estimates presented here can help public health officials to set rational and evidence-based COVID-19 control policies. Note that the proportion of mild cases detected has increased as surveillance and monitoring systems have been strengthened. The incubation period for these severe cases may differ from that of less severe or subclinical infections and is not typically an applicable measure for those with asymptomatic infections

In conclusion, in a very short period health care systems and society have been severely challenged by yet another emerging virus. Preventing transmission and slowing the rate of new infections are the primary goals; however, the concern of COVID-19 causing critical illness and death is at the core of public anxiety. The critical care community has enormous experience in treating severe acute respiratory infections every year, often from uncertain causes. The care of severely ill patients, in particular older persons with COVID-19 must be grounded in this evidence base and, in parallel, ensure that learning from each patient could be of great importance to care all population,

Conflict of interest: The author declares that he has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Appendix

Who is at Higher Risk?

Early information shows that some people are at higher risk of getting very sick from COVID 19. This includes:

- Older adults,with comorbidities
- People who have serious chronic medical conditions like:
  - Heart disease
  - Diabetes
  - Lung disease

COVID-19 outbreak in Italy and it could last for a long time. (An outbreak is when a large number of people suddenly get sick.) , Public health officials recommended community actions to reduce people's risk of being exposed to COVID-19. These actions can slow the spread and reduce the impact of disease.If you are at higher risk for serious illness from COVID-19 because of your age or because you have a serious long-term health problem, it is extra important for you to take actions to reduce your risk of getting sick with the disease.

Everyday precautions

- Avoid close contact with people who are sick
- Take everyday preventive actions
- Clean your hands often

Wash your hands often with soap and water for at least 20 seconds, especially after blowing your nose, coughing, or sneezing, or having been in a public place.

If soap and water are not available, use a hand sanitizer that contains at least 60% alcohol.

To the extent possible, avoid touching high-touch surfaces in public places – elevator buttons, door handles, handrails, handshaking with people, etc. Use a tissue or your sleeve to cover your hand or finger if you must touch something.

Wash your hands after touching surfaces in public places.

Avoid touching your face, nose, eyes, etc.

Clean and disinfect your home to remove germs: practice routine cleaning of frequently touched surfaces (for example: tables, doorknobs, light switches, handles, desks, toilets, faucets, sinks & cell phones)

Avoid crowds, especially in poorly ventilated spaces. Your risk of exposure to respiratory viruses like COVID-19 may increase in crowded, closed-in settings with little air circulation if there are people in the crowd who are sick.

Avoid all non-essential travel

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Natural small molecules as inhibitors of coronavirus lipid-dependent attachment to host cells: a possible strategy for reducing SARS-COV-2 infectivity?

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Abstract: *Background:* Viral infectivity depends on interactions between components of the host cell plasma membrane and the virus envelope. Here we review strategies that could help stem the advance of the SARS-COV-2 epidemic. *Methods and Results:* We focus on the role of lipid structures, such as lipid rafts and cholesterol, involved in the process, mediated by endocytosis, by which viruses attach to and infect cells. Previous studies have shown that many naturally derived substances, such as cyclodextrin and sterols, could reduce the infectivity of many types of viruses, including the coronavirus family, through interference with lipid-dependent attachment to human host cells. *Conclusions:* Certain molecules prove able to reduce the infectivity of some coronaviruses, possibly by inhibiting viral lipid-dependent attachment to host cells. More research into these molecules and methods would be worthwhile as it could provide insights the mechanism of transmission of SARS-COV-2 and, into how they could become a basis for new antiviral strategies.

Key words: Coronavirus, SARS-COV-2, lipid raft, cholesterol, phytosterol

Introduction

Glycoproteins S (spike proteins) of the new coronavirus (SARS-CoV-2) bind to the Angiotensin-Converting Enzyme 2 (ACE2) receptors on human respiratory epithelial cells. During attachment, the S glycoprotein divides into S1 and S2 subunits. S1 contains the receptor-binding domain by which the coronavirus binds to the peptidase domain of the ACE2 receptor. S2 intervenes later, during fusion of the plasma membranes (1). Whole genome sequencing analysis of 104 isolates of the virus causing SARS-COV-2 in patients from different locations showed 99.9% homology, without significant mutations. Almost 80% homology with the SARS-CoV genome and more than 90% with the bat coronavirus genome were also confirmed (2,3). Indeed, the new coronavirus causes a disease that has SARS-CoV-like symptoms. In particular, studies suggest that the SARS-COV-2 receptor that recognizes ACE2 on the target cell membrane is similar to that of

SARS-CoV. SARS-COV-2 nucleocapsid protein has an amino acid sequence identity of almost 90% with SARS-CoV (4).

Most studies on the new SARS-COV-2 are based on previous research carried out on other human coronaviruses in order to identify molecular targets that could be blocked to inhibit entry of the virus into the cell. Coronaviruses are a class of viruses with a long single positive RNA molecule (27-30 kb) and a lipid envelope that requires a plasma membrane fusion process mediated by endocytosis, a mechanism in which cholesterol and lipid rafts play a fundamental role in the early stage of infection of a cell (5,6).

Materials And Methods

This qualitative review is based on data from original documents and reviews. The most pertinent studies on cholesterol and lipid rafts are summarized and interpreted to highlight the role of these membrane molecules in coronavirus infectivity, with a view to their possible consideration in new studies as targets for reducing human infectivity of SARS-COV-2.

We conducted an electronic search in Medline, PubMed and Scopus using different combinations of the search terms "virus", "SARS-COV-2", "coronavirus", "lipid raft", "sterol OR phytosterol" and "cholesterol". This allowed us to link the most recent studies on the infectivity mechanism of SARS-COV-2 with past studies on the role of membrane lipids in the attachment of viruses to host cells. We included studies concerned with the infectivity of types of virus that exploit lipid components of the host cell. The references of all articles were scanned to retrieve any relevant information.

Results

Cholesterol rafts and viral attachment

Cholesterol on the target cell is important for SARS-CoV infection. In the initial stages of a SARS-CoV and Human Parainfluenza virus infection, cholesterol and lipid membrane rafts play a fundamental role in viral entry into the cell (7). The virus attacks these surface molecules on the host cell in specific areas of the plasma membrane characterized by lipid rafts (5). Certain cholesterol-rich microdomains facilitate interaction between the spike protein and its ACE2 receptor (8). SARS-COV-2 is a member of a virus family with a lipid envelope that fuses with the host cell through endocytosis, internalizing its components in the cell (5).

Lipid rafts are important plasma membrane areas for the endocytosis process (5,6), for instance in the early stages of internalization of coronaviruses (9). For example, lipid rafts are involved in the entry of infectious bronchitis coronaviruses. The murine hepatitis coronavirus also requires specific interactions between its spike proteins and lipid rafts on host cells (10,11). This is confirmed by other studies showing that the infectivity of viruses, including coronaviruses, is stimulated by homeostatic control of cholesterol and regulation of fatty acid metabolism (12). *In vitro* experiments show that the sensitivity of the virus to fusion with the host cell membrane increased by cholesterol supplements. For example, cholesterol-supplemented mouse fibroblasts showed increased susceptibility to fusion with murine hepatitis virus (13). There is no significant co-localization of ACE2 with lipid rafts in the plasma membrane of cell models for SARS-CoV infection studies (14). In any case, in lipid raft areas there are also caveolins, clathrins and dynamin, molecules that could have a fundamental role in the internalization of viruses. Internalization mechanisms that depend strictly on these molecules and on the presence of lipid rafts have been described for the simian virus (SV40) (6).

Molecular inhibitors of virus lipid-dependent attachment

Macromolecules such as methyl-β-cyclodextrin (MβCD) and other compounds with depletive cholesterol activity have been used to inhibit attachment of coronaviruses to host cells (15). The lipophilic core allows the interaction of these molecules with lipid rafts (Figure 1). Studied for their antiviral activity, these non-toxic macromolecules mimic attack sites for the enveloped virus, competing with host cell attack sites (16,17). Longer exposure to high MβCD concentrations may also lead to redistribution of cholesterol between raft and non-raft membrane regions (18).

In vitro cell models expressing the ACE2 membrane protein have shown that depletion of cholesterol by MβCD halved the number of bonds with viral S glycoproteins (8). The molecule not only affects cholesterol levels but also expression of the ACE2 receptor. Some studies showed that MβCD treatment slightly and dose-dependently reduced expression of ACE2 in the cell membrane, also reducing the infectivity of coronaviruses, such as SARS-CoV (14).

Other lipophilic molecules, classified as phytosterols, readily interact with the molecules of lipid rafts. This interaction can lead to membrane cholesterol reduction or destabilization of its structure (Figure 2). It could also influence biochemical signaling activities downstream of the rafts (20). These natural molecules are similar in structure to cholesterol. One, extracted from the root of *Aerva lanata*, affects HIV infectivity (21). Other plant sterols, such as β-sitosterol, reduce the probability of HIV and HBV infectivity (22). Řezanka et al. described the antiviral activity of sterols and triterpenoids, molecules which form the basis of many new synthetic drugs aimed at reducing the infectivity of viruses (23). It is well documented that regular phytosterol intake can reduce LDL cholesterol by around 10% (24,25). Betulinic acid is a phytosterol that also has lipophilic properties. Like triterpenes, this compound has a structure similar to cholesterol (Figure 2). It may therefore compete with cholesterol, replacing it in plasma membranes, or it may link to the virus instead of raft cholesterol, acting as a soluble competitor (20). Liposomes are widely used for studying lipid rafts (26). These models have demonstrated that treatment of virus-infected cells with polyunsaturated liposomes has effective antiviral activity in HCV, HIV and HBV infection, reducing levels of cell- and virus-associated cholesterol (27). This treatment could reduce attachment of viruses to host cells.

Discussion and Conclusions

The role of lipids in viral infections suggests cues for understanding the recent SARS-COV-2 infection. Targeting host lipids is already being studied as an antiviral strategy and could have various applications (28).

Cyclodextrins and phytosterols could have health benefits, such as reduction of blood cholesterol levels, and are used to prevent and reduce the risk of coronary disease, to reduce inflammation, to induce apoptosis in cancer cells and to treat viral infections. Today these natural compounds are also available in different forms as supplements. The aim of the present review was to offer a panorama of the role of these molecules in one of the many mechanisms involved in the entry of viruses into host cells. These molecules and their antiviral properties should be borne in mind in double

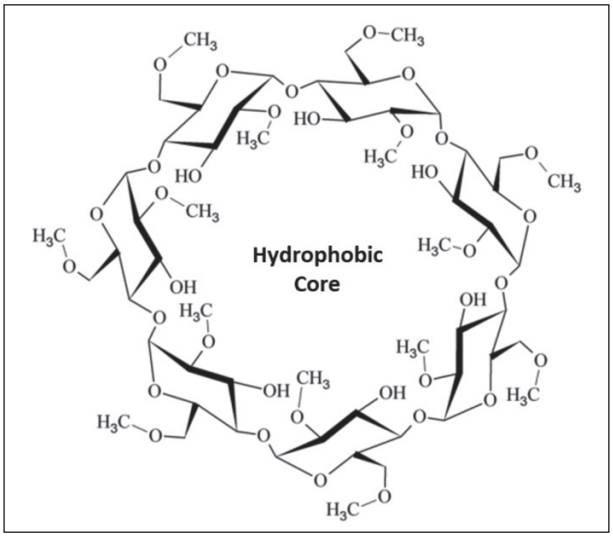

Figure 1. Molecular structure of a methyl-β-cyclodextrin. Adapted from Fenyvesi et al. (19)

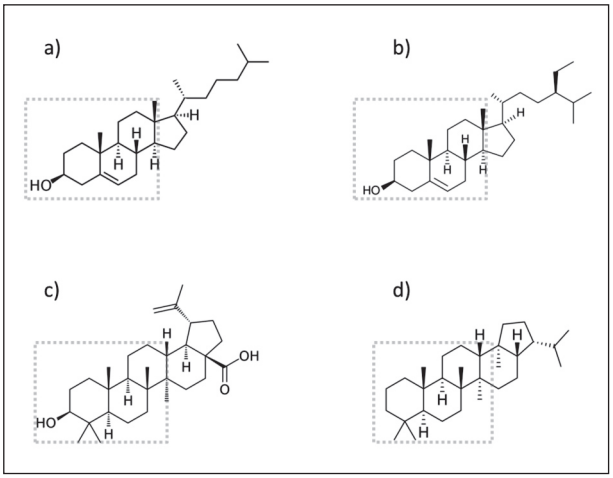

Figure 2. Molecular structure of cholesterol (a), β-sitosterol (b), betulinic acid (c) and hopane, an example of a pentacyclic triterpene (d). Similarities in core structure are marked by dashed line.

blind trials concerning the SARS-COV-2 pandemic, testing them in a cohort of volunteers and patients and reporting any evidence of their ability to reduce viral infectivity.

Conflict of interest: The author declares that he has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

Current approach to the clinical care of adolescents with gender dysphoria

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Summary. Over the last decade, we have witnessed a significant rise in the number of transgender young people seeking endocrine treatment, of which clinical service and gender dysphoria terminology have attempted to keep pace both in matching demand and better describing the condition. Although helpful guidelines for pubertal suppression and gender affirming hormones have been developed, uncertainties remain regarding treatment and monitoring during treatment, often because the clinical needs of the transgender population have outpaced medical expertise and training. Recently, multidisciplinary team work has evolved due to the increasing complexity of diagnostic and treatment decision-making and has been instrumental in creating a unique service with input from a range of specialists. In this article, the current approach in clinical management of adolescents with gender dysphoria is reviewed, with focus on the endocrine aspect of care in children and adolescents. Questions on what defines optimal clinical care of children and adolescents with gender dysphoria remain and should be the focus of future research. (www.actabiomedica.it)

Key words: gender dysphoria, transgender, adolescents, Gn-RH analogue, gender affirming hormones

Introduction

Over the last decade we have witnessed a rapid evolution in terminology in gender dysphoria (GD), initially termed gender identity disorder, then gender dysphoria (DSM-V), and, most recently, gender incongruence (ICD-11). Gender incongruence is characterised by a marked and persistent incongruence between an individual's experienced gender and their assigned sex. Gender variant behaviour and preferences alone are not a basis for making the diagnosis in this group (1). The changes in nomenclature reflect changes in opinion within the medical profession and the public domain as well as the influence of individuals with GD.

Expert professionals working in specialist centres have moved away from definitions relating to psychiatric disorders, which has aided demedicalisation in GD. Currently, the term GD is most commonly used and refers to the distress that may accompany the incongruence between one's experienced or expressed gender and one's assigned sex at birth (2).

The terminology applied to GD can be confusing for non-specialists but is of fundamental importance in the description and our understanding of the clinical presentation. Biological sex, gender identity, and gender expression should be considered separately so as to best comprehend the whole.

Biological sex is determined by the karyotype (classically, 46,XX in females and 46,XY in males)

and the reproductive organs of the individual. Gender identity is the inner understanding and perception of oneself as a man or a woman or anything along the spectrum between man and woman. Gender expression is how we express our gender on the male to female scale and it is influenced by culture and norms in society (3, 4). The expected perception is that all three terms should be in agreement with one another in any one individual; however, there can be circumstances in which this expectation is not manifested in reality.

In GD, the person's gender identity and biological sex do not match, resulting in marked distress, which is often heightened at onset of puberty with the associated development of secondary sex characteristics (5). The distress can be so debilitating as to hinder normal psychosocial development and activities of daily living, often resulting in depression and suicidal ideation. Access to treatment, including psychotherapy, hormones, and surgery, improves prognosis (3).

Clinical presentation

Children may express a dislike of their sex characteristics and a desire for the characteristics of the gender they identify with. Cross-dressing, cross-gender roles in play, preference for toys and activities, friends of the opposite gender, and rejection of cultural gender roles are all commonly reported and are used as diagnostic criteria for GD (5,6). Difficulties in childhood often arise from social intolerance and poor social relations with peers, as well as negative psychological outcome in children with GD (7). A cross-national study carried out in Canada and the Netherlands in children with GD showed more emotional problems and poorer peer relations in the former country than in the latter, suggesting that individuals fair better in those societies that are more tolerant. Good peer relations were of more importance than IQ, social status, marital status of parents, and ethnicity as predictors of positive emotional outcomes in children with GD (8).

Importantly, young children may or may not continue to identify themselves as transgender in adolescence and adulthood. Indeed, gender incongruence will desist by early adolescence in the majority. Several studies have shown that the percentage of persisters lies between 10 and 39% (6, 9), when gender variance presents in childhood, and that the critical time period for GD persistence or desistence is between the ages of 10 and 13 years (9). Important factors that have been associated with persistence of GD in adolescence/adulthood include the intensity of GD, the persistence, insistence, and consistence of statements, and behaviours in childhood, as well as a strong tendency to report their gender (10).

More recently, expert professionals have seen an ever-increasing number of post puberty cases of GD in birth-assigned females with rapid-onset clinical manifestations. This apparent new phenomenon, termed "rapid-onset gender dysphoria" (ROGD), has been described by parents who have reported that their child displayed a sudden or rapid onset of GD in adolescence without having had a history of gender variance during childhood (11, 12). Of note, clinical features suggestive of GD were observed in adolescents within a group of peers, with several members becoming gender nonconforming. A survey of 256 parents showed that the majority of adolescents with ROGD were birth-assigned females (82.8%), with a mean age of 16.4 years. In addition, there were a high percentage of mental health disorders and developmental disorders, as well as several psychosocial stressors, which preceded the onset of GD. The survey received mixed support, and warrants future studies to help understand if ROGD as a distinct entity or as a variant presentation of GD (12-15).

Comorbidities in young people with GD

Mental health problems remain one of the major co-existing concerns in transgender young people. Anxiety, eating disorders, depression, self-harm, and suicidal ideation have been well-documented in adolescents and adults with GD (16, 17). Mental illness in transgender individuals seems to be multifactorial, with contributions from any of social rejection, stigma, discrimination, low access to health care providers with expertise in transgender health, and limited availability to multidisciplinary team of experts (18). Comorbidity studies in children and adolescents have found a high prevalence of autism spectrum disorder (ASD) traits or

confirmed diagnosis of ASD in gender dysphoria. In addition, adults with gender dysphoria attending specialist gender clinics have also been shown, to exhibit autistic traits, as indicated by high social responsiveness scores (indicating autistic features) and have higher rates of ASD diagnosis compared to the general population (19-22). Violence and victimisation, inclusive of sexual assault, dating violence and bullying are common encounters for young individuals with GD (23).

Epidemiology of GD

The prevalence of GD according to published reports is highly variable. Studies in children and adolescents have shown a prevalence of GD ranging between 1.2 and 2.7%, although a disconcertingly high percentage of respondents revealed that they did not understand the question (24-26). In DSM-V, the prevalence of GD in adulthood for birth-assigned males ranges between 0.005 and 0.014% and for birth-assigned females between 0.002 and 0.003%. These published prevalence rates are assumed to underestimate true prevalence, as there is ascertainment bias in how these data are collected due to the inclusion only of those individuals who seek treatment in a specialist centre. Individuals will not be captured if they do not seek treatment or if they receive treatment outside of a specialist centre. This may in part be circumvented by studies using self-reporting in which prevalence rates of 0.5-1.3% have been documented (27)adolescents and adults. Although the prevalence of gender dysphoria, as it is operationalised in the fifth edtion of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5.

There are geographical differences in the prevalence of GD, which in part relate to cultural norms and also differences in diagnostic criteria internationally. In addition, over the last decade there has been a significant increase in individuals with GD presenting to specialist clinics (28, 29). Several explanations have been proposed, including change in help-seeking attitude, raised public awareness along with increased media presentation, the internet as a source of information, LGBT support groups, campaigns for transgender rights, reduced discrimination, greater awareness of GD among healthcare professionals, and advances in understanding the aetiology of GD (30).

In addition to the epidemiological changes, there has been a shift in the sex ratio among individuals with GD towards more presentations in birth-assigned females. Birth-assigned males to birth-assigned females ratio was 2.1:1 for the years 1999-2005 in Toronto, whereas the sex ratio changed to 1:1.8 from 2006 to 2013 (31).

Subsequent studies have similarly illustrated a clear shift in the sex ratio favoring birth-assigned females (28, 29, 32). However, since the true prevalence of GD is unknown, safe conclusions cannot as yet be extracted.

Even though it is clear that the number of children and adolescents with GD who seek professional help is rising, recent findings suggest that the exponential increase in referrals might reflect that seeking help for gender dysphoria has become more common rather than that adolescents are referred to gender identity services with lower intensities of gender dysphoria or more psychological difficulties, as the young people did not show critical changes in key demographic, psychological, diagnostic, and treatment characteristics over 16 years, with the exception of the shift in sex ratio (32).

The staged approach in clinical management

Multidisciplinary approach and clinic environment

Treatment protocols for use in individuals with gender dysphoria should be designed with an aim of fostering a good relationship with young people and their families, addressing their needs in order to achieving satisfactory outcomes (2). The two main outcomes of gender dysphoria treatment are to support a young person's transition, aligning the phenotype with the experienced and/or expressed gender identity, and to support their psychosocial wellbeing. These outcomes are interlinked and are better achieved using a multidisciplinary approach, involving both physical and nonphysical interventions.

A child or young person with gender dysphoria should be referred to a specialist centre with mul-

tidisciplinary support (33, 34). Multidisciplinary team (MDT) work for GD management has evolved due to the increasing complexity of diagnostic and treatment decision-making. An MDT approach aims to bring together the range of specialists required to discuss and agree treatment recommendations and ongoing management. The multidisciplinary clinic as a minimum should be comprised of a core group of specialists including paediatric and adult endocrinologists, clinical psychologists, psychiatrists, and nurse specialists.

It is important that the core group agree a unified approach to management and that each specialist communicate with families using similar language. Specialists working in the MDT will recognise the importance of sharing information within the team and providing clear information to young people with GD. The high prevalence of young people presenting with co-morbid ASD and autistic spectrum traits requires special recognition and the MDT should consider attaining additional skills in communicating in this area.

Young people accessing the MDT clinic may feel disenfranchised from their family and community. It is very likely that individuals with GD will have experienced stigmatisation and discrimination in different settings including home, local community and school. It is therefore important that young people who have reached the MDT service should feel secure that the clinic represents a space where they can be secure of confidentiality and without judgement (3).

Treatment options are tailored to the individual but will be guided by the age of the person and staging of puberty, the birth-assigned sex, and capacity to provide consent, among other factors.

Stage 1: ***Psychology Support***

To date no single or combination of parameters has allowed clinicians to clearly differentiate between children who will show persistence of gender-variant behaviour in adulthood from those who show desistence and conform to their natal gender. Treatment in prepubertal children with gender dysphoria remains contentious, as, relative to adolescents and adults, they are less likely to express a stable pattern of gender variance, with the majority not having GD by onset of puberty (34). The general approach in a prepubertal child with gender variance would be to offer the family similar access to support networks whilst allowing for the developmental trajectory of gender identity to unfold without pursuing or encouraging a specific outcome and the avoidance of taking any irreversible steps (34). The adoption of this treatment path does not exclude active support of the child's social integration and wellbeing, in order to minimise social risks and stressors, whilst behavioural, cognitive and emotional coping strategies can be promoted (18, 34).

Stage 2: ***Suppression of puberty***

During puberty, adolescents who fulfill the criteria for suppression of puberty (see below) will be referred to a paediatric endocrinologist for discussion of medical interventions to delay pubertal progression. The use of Gonadotrophin Releasing Hormone (Gn-RH) analogue to suppress puberty followed by introduction of gender affirming hormones in later adolescence was first described in Amsterdam, The Netherlands, in the 1990s (35).

The use of Gn-RH analogue is deemed appropriate in those young people with persistence of GD beyond the onset of puberty (Tanner stage 2: initiation of breast development in birth-assigned females, testicular volume >4 mL in birth-assigned males). The mechanism of action of Gn-RH analogue therapy is through desensitisation of the Gn-RH receptor and, in turn, suppression of gonadotrophin release; therefore, it stops puberty and halts further development of secondary sex characteristics.

The treatment with Gn-RH analogue in adolescents with GD, based on the existing evidence, is both effective and sufficiently safe (36); however, adolescents should fulfill certain criteria to be eligible for pubertal suppression. According to the Endocrine Society, treatment with Gn-RH analogues should be proposed if:

(i) GD has been diagnosed, based on clinical criteria;

(ii) initiation of puberty has been confirmed and contraindications to Gn-RH analogue treatment do not exist;

(iii) the adolescent and their parents have been fully informed about the effects, the side effects, and

the impact of the treatment on future surgical procedures, as well as about the fertility preservation possibilities;

(iv) the adolescent has fully understood the treatment protocol and has given their informed consent/assent; and

(v) pubertal suppression is proposed by an MDT with expertise in transgender health (2).

Gn-RH analogues are administered by intramuscular or subcutaneous injections, 4-weekly, or 12-weekly (37). A newer formulation, administered 24-weekly, was approved in 2017. The use of Gn-RH analogue in GD is considered off-label.

Through stopping pubertal progression, Gn-RH analogue helps children with established GD to alleviate their distress and anxiety, which are both linked to appearance of secondary sex characteristics (3, 34). Halting progression of puberty improves behavioural and emotional problems and reduces depressive symptoms. Thus, Gn-RH analogue can provide a breathing space for the young person to explore their gender identity with the support of their mental health professional prior making decisions on treatments associated with irreversible change. During this time, young people will be encouraged to take the opportunity to obtain real-life experience living as the non-assigned gender in dress and behaviour, and determine whether or not they desire full transition. In addition, GnRH analogue may prevent further development of unwanted secondary sex characteristics, obviating the need for future affirming surgeries and making it easier for the person to live in their affirmed gender in the future (34, 38). Global psychosocial functioning was improved significantly in 201 adolescents with GD after 12 months of suppression of puberty with Gn-RH analogue (39).

Possible unwanted effects and uncertainties

Discontinuing treatment will lead to the re-activation of the pituitary-gonadal axis; in that respect, the effects of Gn-RH analogue are considered completely reversible. Side effects include redness and swelling reported by 9% of young people and local pain in up to 10-20% (40). In addition, mood changes, worsening acne, vaginal bleeding, vaginal pain and itching, and fewer erections have been reported in young people receiving pubertal suppression (36, 40, 41). Side effects of Gn-RH analogue are consistent with the physiological effects of hypogonadism, such as vasomotor instability and hot flushes, headaches and emotional lability, and mood disturbance.

Puberty is the most important period in life regarding the accumulation of bone mass. In general, about 85-90% of the total bone mass will have been acquired at the end of puberty. Sex steroids reach high concentrations as puberty progresses and play a key role in the bone growth and bone mass accumulation. It is not well understood how the suppression of puberty with Gn-RH analogue affects the development of peak bone mass and bone mineral density (BMD), although some studies with small cohort sizes have found that BMD Z-scores are decreased (42, 43).

It is necessary to establish the clinically significant changes that would trigger changes in medical management. Reduction in BMD Z-scores and alterations in body composition (decrease in lean mass and increase in fat mass) may be expected transient effects of suppression of puberty; discontinuation of Gn-RH analogue or initiation of gender affirming hormones are expected to correct those changes. However long-term studies of bone health in young people receiving Gn-RH analogues are as yet not available, and, until further studies are conducted on bone health, this conjectured catch-up of bone accrual on cessation of Gn-RH analogue will remain an assumption.

Uncertainties also exist regarding the effect of puberty suppression on growth and adult height, the psychosocial problem of delayed puberty and possible effects on brain development (44-47). Continuing to support future research on the effects of Gn-RH analogue is essential, whilst delivering clinical service to young people (48).

Monitoring during treatment with Gn-RH analogue

Monitoring should focus on achieving the goals of treatment as stated above, while preventing or identifying unwanted side effects (table 1). During treatment, young people should be reviewed by a paediatric endocrinologist at a minimum frequency

Table 1. Suggested clinical, biochemical and imaging assessments before and during treatment with GnRH analogue

Assessments prior and during treatment with Gn-RH analogue	
Prior commencing Gn-RH analogue	
Height, height velocity, weight, BMI	
Bone age (in those who have not completed puberty)	
Pubertal assessment	
Blood pressure	
Haemoglobin/Haematocrit, ferritin	
Liver function, renal function, electrolytes	
LH, FSH, oestradiol/testosterone	
Prolactin	
Vitamin D, PTH, calcium, phosphate, albumin	
BMD (and VFA) by DXA	
During treatment with Gn-RH analogue	
Height, height velocity, weight, BMI	Every 3-6 months
Bone age	If clinically indicated
Pubertal assessment	Every 6 months (if possible)
Blood pressure	Every 3-6 months
LH, FSH, oestradiol/testosterone	Every 6 months
Vitamin D, PTH, calcium, phosphate, albumin	Every 6 months
BMD (and VFA) by DXA	Every 12 months

Legend: GnRH: Gonadotrophin Releasing Hormone, BMI: Body Mass Index, LH: Luteinising hormone, FSH: Follicle stimulating hormone, PTH: Parathyroid hormone, BMD: Bone mineral density, VFA: Vertebral Fracture Assessment, DXA: Dual Energy X-ray Absorptiometry.

of every 6 months. The efficacy of treatment is confirmed by slow height velocity and the halt of pubertal progression identified by clinical assessment. Biochemical suppression of the pituitary-gonadal axis is indicated by a significant reduction in plasma gonadotrophins, albeit not always to prepubertal levels. At all times during treatment, the MDT are required to ensure that suppression of puberty improves the distress, anxiety and psychosocial functioning of the young people. If this is not the case, the MDT should review the treatment plan and, on consultation with the young person, consider discontinuation of Gn-RH analogue when the expectations of the treatment are not met. In terms of bone health, adolescents should be encouraged to make healthy lifestyle choices and improve their physical activity levels, focusing on weight bearing exercise.

Bone biochemistry, including vitamin D, should be assessed at regular intervals and actively supplemented in insufficient and deficient states. BMD by dual energy X-ray absorptiometry should be assessed.

Stage 3: ***Gender-affirming hormones***

In adolescents willing to proceed via hormonal transitioning, the treatment involves the use of gender-affirming hormones, (estrogens for trans-females and testosterone for trans-males), aiming for the development of secondary sex characteristics of the affirmed gender. Importantly, the Endocrine Society does not specify a minimum age for hormonal treatment; The initiation of gender affirming hormones will be consider after an MDT of medical and mental health professionals has confirmed: (i) the persistence

of GD (ii) the absence of psychological, medical or social problems that may interfere with treatment (iii) the ability of the person to understand the benefits and risks of therapy (including the irreversible changes in their body, the detrimental impact on fertility and possible side effects) and can consent to this treatment (2).

Before starting sex hormone treatment, effects on fertility and options for fertility preservation should be discussed.

The current consensus on age of initiation of sex hormone treatment is 16 years (2, 33). Potential risks of waiting until age 16 years include those to bone health, if puberty is suppressed for many years before initiating sex hormones, and to emotional and social isolation if lack of secondary sex characteristics is causing distress. However, only minimal data and clinical experience supporting the use of gender-affirming hormones in transgender adolescents at a younger age currently exist (29,33). Long-term studies are needed to determine the optimal age of sex hormone treatment.

Hormonal treatment should be initiated progressively (pubertal induction), with the dose increasing gradually, and should occur in parallel with psychological monitoring. For the induction of puberty in GD, clinicians can use a similar schedule to those in hypogonadism, closely monitoring for desired and unwanted outcomes. Suggested assessments during induction of puberty are summarised in table 2.

In transgender males, during pubertal induction with testosterone, the initial levels will not be high enough to suppress endogenous sex steroid secretion. Thus, Gn-RH analogue treatment should continue until an adult dose of testosterone has been reached. In transgender females continuation of Gn-RH analogue treatment is recommended until gonadectomy, because gonadotrophins and endogenous production of testosterone will interfere with the efficacy of estrogen supplementation (49, 50).

In those who may decide not to have gonadectomy, prolonged Gn-RH analogue treatment is an option, however the potential risks of this treatment are currently unknown. Alternatively, transgender females may be treated with an anti-androgen that directly suppresses androgen synthesis or action.

Stage 4: ***Gender affirmation surgery***

After an agreed upon time (known as social gender role transition) during which the person will live according to their identified gender, and beyond the age of 18 years, the option for gender-affirming surgery is offered. However, the World Professional Association for Transgender Health Standards of Care states that the threshold of 18 years should not be seen as an indication in itself for active intervention. If the social transition has not been satisfactory, if the person is not satisfied with or is ambivalent about the effects of sex hormone treatment, or if the person is ambivalent about surgery then a referral for surgery should not be made (2, 51).

The most common surgical procedures performed in trans-males include mastectomy, and genital surgeries including salpingo-oophorectomy, hysterectomy, and creation of neopenis with implantation of erectile and testicular prostheses. In trans-females, surgical procedures include breast augmentation surgery, facial feminisation surgeries and thyroid cartilage reduction and genital surgeries: prevalent techniques include gonadectomy, penectomy, and creation of a neovagina.

Surgeons invert the skin of the penis to form the wall of the vagina and the scrotum becomes the labia majora. The timing of initiation of Gn-RH agonist therapy in birth-assigned boys relative to pubertal stage has an impact on future options for surgery and should be discussed with families prior to starting treatment.

Both starting Gn-RH analogue early in puberty and prolonged hormonal intake can together culminate in inadequate penile length and scrotal hypoplasia, making penoscrotal inversion vaginoplasty not feasible, thus leading to more complex surgical techniques. Young people and their families should be duly informed prior to starting treatment, especially at an early pubertal stage. More detailed approach to gender affirmation surgery is outwith the scope of this review.

Impact of medical interventions on fertility and fertility preservation

Loss of fertility incurred as a consequence of hormonal treatment requires consideration before initia-

Table 2. Suggested clinical, biochemical and imaging assessments before and during treatment with gender affirming hormones

Assessments prior and during treatment with gender affirming hormones	
Prior commencing gender affirming hormones	
Height, height velocity, weight, BMI	
Bone age (in those who have not completed puberty previously)	
Pubertal assessment	
Blood pressure	
Haemoglobin/Haematocrit	
Liver function, renal function, electrolytes	
LH, FSH, oestradiol/testosterone	
Prolactin	
Lipid profile	
Vitamin D, PTH, calcium, phosphate, albumin	
BMD (and VFA) by DXA	
During pubertal induction with gender affirming hormones	
Height, height velocity, weight, BMI	Every 3-6 months
Bone age	If clinically indicated
Pubertal assessment	Every 6 months (if possible)
Blood pressure	Every 3-6 months
Haemoglobin/Haematocrit	Every 6 months
Liver function	Every 6 months
Lipid profile	Every 6 months
Prolactin (in transgender females)	Every 12 months
LH, FSH, oestradiol/testosterone	Every 6 months
Vitamin D, PTH, calcium, phosphate, albumin	Every 6 months

Legend: GnRH: Gonadotrophin Releasing Hormone, BMI: Body Mass Index, LH: Luteinising hormone, FSH: Follicle stimulating hormone, PTH: Parathyroid hormone, BMD: Bone mineral density, VFA: Vertebral Fracture Assessment, DXA: Dual Energy X-ray Absorptiometry

tion of treatment. The discussion with young people and their families will be influenced by the stage of puberty, the birth-assigned sex, and the degree of GD, and the availability and acceptance of assisted reproductive technologies (ART).

Before starting treatment with Gn-RH agonist therapy, sperm and oocyte retrieval and banking can be offered to those who are postpubertal. In birth-assigned males, at Tanner stage 3, ejaculation or electroejaculation can take place and yield sufficient sperm for preservation. In birth-assigned females, oocyte harvesting is only available if they are post-menarchal.

Young people who commence treatment with Gn-RH analogue at Tanner stage 2 and continue on to gender-affirming hormones will achieve neither spermatogenesis nor menarche and will therefore not have the opportunity to bank gametes using cryopreservation. If individuals subsequently want to preserve fertility after having started Gn-RH analogue, it may take 6 months or more for the reproductive axis to recover and the reproductive capacity will only be the same as at the point of starting treatment. Nevertheless, many young people and families, after appropriate informed consent, opt not to proceed with fertility preservation.

The current experiences of trans people with fertility preservation services are mostly negative. With the anticipated advances in methods for fertility preservation, it is essential to identify the barriers the transgender young people face and make the service easier to approach, taking into account the right of transgender people to procreate (3,28,52).

Conclusions

The number of transgender young people seeking endocrine care has increased over recent years, in part related to increasing social acceptance and destigmatisation of GD. During the last decade, we have witnessed tremendous progress in terminology and evolution of specific diagnostic criteria, and increasing numbers of clinical specialist centres. Guidelines for treatment with Gn-RH analogue and gender-affirming hormones are now available; however, a number of uncertainties still exist. Larger studies are clearly required to delineate the positive outcomes in psychosocial functioning and quality of life and the long-term effects of pubertal suppression and gender-affirming hormone therapy on metabolism, on the growing skeleton, and on brain development and cognition.

It is clear that a multidisciplinary expert team cognizant of the complexities of GD, including co-existing mental health and communication problems, are of primary importance in developing a supportive environment for young people and their families.

Clinicians should be supported by health boards to deal with the uncertainties they face. In the meantime, it remains the responsibility of the team to continue to review and develop the GD service to inform future service development whilst matching the needs of young people and ensuring goals of treatment are met.

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Review

Striae distensae in adolescents: a mini review

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Summary. Striae distensae or stretch marks are mainly a cosmetic concern. They commonly occur in adolescence and in pregnant women. Although, generally more common in females; physiological striae atrophicae of adolescence are more common in males. The pathophysiology is multifactorial with mechanical stretching of the skin being the most important. Despite of an abundance of treatment modalities none is 100% effective. (www.actabiomedica.it)

Key words: adolescents, striae distensae, stretch marks

Introduction

Striae distensae (SD) were first described in the medical literature by Troisier and Menetrier in 1829. In 1936, Nardelli named the lesions striae atrophicae (1).

SD or stretch marks result from dermal scarring and epidermal atrophy. The epidermis is thin with loss of dermal papillae and rete ridges and the dermis shows a decrease in extracellular matrix (ECM) components; collagen, fibronectin, fibrillin and elastin (2-4).

The dermis is composed of an interwoven matrix of collagen and elastin (3). In normal skin collagen fibrils are organized in densely packed bundles that provide support to the skin (5). Elastic fibres allow the skin to stretch and return to its original shape (6). With development of SD, the collagen bundles separate and collagen fibrils fail to form bundles. Elastic fibres are disrupted and tropoelastin (soluble elastin)-rich fibrils unable to organize into normal-appearing elastic fibres form (5).

Stretch marks are caused by excessive mechanical stretching of skin to the point of rupturing dermal elastic fibers with local fibroblasts unable to adequately repair or replace ECM components (4). Aberrant fibroblast function may be responsible for development of SD as fibroblasts from striae expressed significantly less fibronectin and both type I and type III procollagen (7).

Clinically, the condition passes through two stages: an initial raised erythematous, inflammatory stage (striae rubrae; SR) and a white, depressed, finely wrinkled second stage (striae albae; SA) (8).

Prevalence, etiology and risk factors

In the adolescent population reported prevalence ranges from 6% to 86%. In adolescent males the buttocks, lower back and knees are usually affected while in females the buttocks, thighs and calves are more often involved (9).

Three main theories underlying the development of SD are described: mechanical stretching of the skin, hormonal changes and an innate structural disturbance of the skin (9). SD are postulated to result from an initial inflammatory reaction that destroys collagen and elastic fibers, followed by the regeneration of collagen and elastic fibers in the direction imposed by mechanical forces (10).

Genetic factors may be operative as a familial form of striae was described by McKusick (11). Also striae in monozygotic twins and striae in syndromes as Ehlers-Danlos, Marfan and ectodermal dysplasia have

been reported (12). Results of a genome wide association analysis support the hypothesis that variations in the elastic fiber component of the skin extracellular matrix contribute to the development of stretch marks (13).

Physiological striae atrophicae of adolescence occurs mainly in healthy, nonobese individuals at around puberty in association with the adolescent growth spurt (14). It commonly occurs in the gluteal region, breasts, thighs, lower abdomen and back (15). SD associated with pubertal growth spurt becomes less conspicuous with time and has excellent prognosis as compared to other SD (16).

In adolescents, high BMI, obesity during childhood, and facial seborrhea correlate positively with development of SD. Striae have also been observed in conjunction with Cushing syndrome and exogenous steroid use (17). To investigate the role of hormones in the development of SD, the expression of estrogen receptor (ER), androgen receptor (AR) and glucocorticoid receptor (GR) in SD was studied. Cordeiro et al. (18) found 2.2-fold more ER, 1.8-fold more AR and 1.7-fold more GR in SD samples compared to normal skin (9). These results were supported by other studies, however, one study found reduced ER β expression in SD lesions and perilesional normal skin compared to a control group despite the presence of increased expression of both AR and GR.

Hormone receptor expression is increased under certain conditions suggesting that regions undergoing greater mechanical stretching of the skin may express more hormone receptor activity thus influencing the metabolism of the extracellular matrix, causing SD formation. This could be the link between hormonal and mechanical theories underlying the development of SD (19).

ACTH has a catabolic effect on fibroblasts with a resulting decrease of mucopolysaccharides in collagen tissue. Elevated serum levels of steroid hormones (or of their metabolites) have been found in people with striae (1). Striae formation resulting from the use of topical steroids seems to be related to the use of the more potent preparations. Adolescents and young adults seem to be particularly prone to this form of striae formation (20).

Evaluation of striae distensae

A numerical scoring system for the severity of striae was devised for the evaluation of striae gravidarum. The number of striae present at different sites (Figure 1) and the degree of erythema were evaluated. At each site striae were scored up to a maximum of six; 0-3 for number of striae present and also 0-3 for the degree of erythema. The number of striae was recorded as: no striae, 0; <5 striae, 1; 5-10 striae, 2; and >10 striae, 3. The degree of erythema was recorded as: no erythema, 0; mild erythema (light red or pink) (Figure 2), 1; marked erythema (dark red), 2 (Figure 3); and violaceous erythema (purple), 3 (Figure 4). The following sites are evaluated: abdomen, hips, breasts, thigh/buttocks with a maximum score of 24 (21).

Management of striae distensae

Many therapeutic modalities are available but none can completely eradicate SD: laser, light therapy, acid peel treatments, collagen injection, laser lipolysis, radiofrequency techniques and microdermabrasion (22). The majority of treatments aim to increase colla-

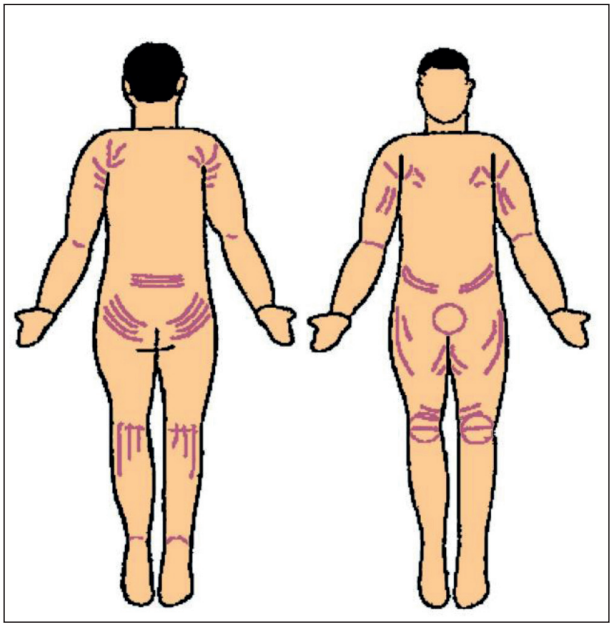

Figure 1. Typical distribution of striae distensae (from: Cho S et al. J Eur Acad Dermatol Venereol. 2006;20:1108-13; modified)

Figure 2. Striae distensae type 1

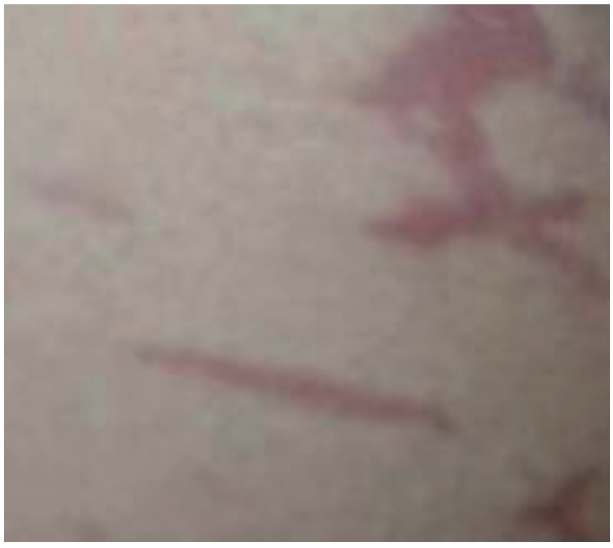

Figure 3. Striae distensae type 2

Figure 4. Striae distensae type 3

gen production, reduce erythema, or increase pigmentation (23).

1. **Enhanced collagen production**

- Tretinoin and retinoic acid are believed to act by stimulation of fibroblasts leading to increase tissue collagen I levels. Side effects include transient erythema, scaling and itching or a burning sensation (24, 25).
- Centella asiatica is a medicinal herb thought to increase the production of collagen and elastic fibers (26). No side effects were observed with its use, however, when combined with boswellic acid which has an anti-inflammatory action, pruritus was reported (23).
- Hyaluronic acid is also suggested to stimulate fibroblast activity and the production of collagen (22).
- Chemical peels are divided into superficial, medium-depth and deep subtypes based on the depth of their penetration (27). For striae distensae 20% glycolic acid and trichloroacetic acid (TCA) 10-35% are also reported to stimulate collagen production by fibroblasts (7). Superficial peels target the epidermis and the epidermal-dermal interface causing partial or complete necrosis. They exfoliate the skin from the stratum corneum down to the papillary dermis at a depth of 60 μm (27).
- Aluminum oxide microdermabrasion induces epidermal signal transduction pathways that are associated with remodeling of the dermal matrix. It produces epidermal and dermal changes through superficial wounding (28).
- Bipolar radiofrequency (RF) devices generate heat in response to poor electrical conductance according to Ohm's law (heat generation is di-

rectly correlated with tissue resistance). The heat generated is responsible for the partial denaturation of pre-existing elastic fibers and collagen bundles. Initial collagen denaturation causes immediate tissue contraction; subsequent neocollagenesis further tightens the dermal tissue and reduces striae. Autologous platelet-rich plasma (PRP) can be injected using the needle electrode of the intradermal RF device as the delivery route. At sites of tissue damage, platelets are the first cells to arrive and through the release of growth factors from their a-granules act on endothelial cells, erythrocytes, and collagen thus aiding in the healing of localized chronic inflammation believed to be a factor in the etiology of striae distensae (29).

- Fractional lasers (FL): produce small columns of thermal injury to the skin, which are known as microthermal zones (MTZs). Some cause nonablative (leave a functionally and histologically intact stratum corneum) dermal injuries only; whereas, others are associated with ablative changes in the skin, causing both epidermal and dermal injuries. MTZs also vary in their diameter and depth. Once injured, the skin begins a very rapid process of repair. The rapid healing process is made possible through the help of the surrounding normal or untreated skin (30). When compared with nonablative lasers, ablative lasers are less well-tolerated and produce inconsistent results (23). A similar technique is microneedling which is a minimally invasive procedure that uses fine needles to puncture the epidermis. The microwounds created stimulate the release of growth factors and induce collagen production. The epidermis remains relatively intact, therefore helping to limit adverse events (31).
- Galvanopuncture, a needling procedure that uses continuous direct microcurrent as an alternative to induce a local inflammatory process intended to repair the affected tissue. The microamperage galvanic current reaches intensities between 50 and 200 μA. It induces modification of the vasculature with dilation of blood vessels, tissue edema, and associated redness. The outcomes are angiogenesis, cell proliferation, as well as reorganization of collagen bundles. The best outcome was observed in subjects with darker skin, which is usually the most difficult skin type (32).

2. Reduction of vascularity

- Lasers with wavelengths of 585 to 595 nm are used, due to a high absorption by haemoglobin and decreased absorption by the competitive chromophore melanin, thereby reducing injury to the epidermis. Longer wavelength lasers (alexandrite laser 755 nm, Nd:YAG laser 1064 nm) have been developed to target oxy- and deoxyhaemoglobin which have the advantage of deeper tissue penetration (33). The treatment of erythematous striae using the 1064-nm long-pulsed Nd:YAG laser demonstrated clinical improvement of such lesions, probably due to the laser's affinity toward the vascular target present in the striae. The absorption of the laser by oxyhemoglobin, leads to an improvement in the redness. In addition, like other luminous sources, the long-pulsed Nd:YAG laser also induces the formation of new collagen (34).

3. Increase melanin production

- A targeted narrow band UVB/UVA1 therapy caused 51% improvement in SA pigmentation after weekly (maximum 10 weeks) phototherapy sessions. Transient hyperpigmentation of striae was seen in almost half the subjects as an adverse event. Skin biopsy failed to show any effect on collagen remodeling, thus limiting its efficacy only for repigmentation of SA (35).
- The 308 nm xenon chloride (XeCl) excimer laser has a wavelength close to that of traditional narrow band ultraviolet B (UVB) light. It causes temporary repigmentation and improvement of leukoderma in SD. Post laser biopsies showed greater melanin content and hypertrophy of the melanocytes, although it failed to show any improvement in skin atrophy (36).

Conclusion

SD or stretch marks are a relatively common skin condition that occurs frequently in association with the adolescent growth spurt and pregnancy. Their etiology is still not completely established. The most promising treatment modality is laser therapy.

Conflict of interest: Each author declares that he or she has no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article

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Review

Prevalence, attitude and practice of self-medication among adolescents and the paradigm of dysmenorrhea self-care management in different countries

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Summary. Self-medication (SM) is an important worldwide public health issue affecting children and adolescents. The pattern of SM varies in different communities, affected by factors such as age, sex, income, expense, self-care orientation, educational level and medical knowledge. It is a fairly common practice: for minor health problems, it often provides cheap, rapid, and convenient solutions, outside of the health care system of many countries. Painkillers, antipyretics, cough medicines, cold preparations, dermatological products, nutritional supplements and antibiotics are the drugs most frequently used. Potential risks include incorrect self-diagnosis, improper dosage, inappropriate choice of therapy, masking of severe disease and drug interactions. Lack of awareness of warnings and precautions, storage conditions, the recommended shelf-life and adverse reactions increase the risk of side effects. Little is known about the SM of dysmenorrhea by adolescent girls. Attitudes towards treatment are influenced by cultural, ethnic, and religious factors. Some girls discuss dysmenorrhea with family and friends, and the majority may not seek medical advice. As dysmenorrhea is a common problem for adolescents, it is essential that these girls be aware of the normal and abnormal symptoms of menstruation. In the light of these findings, the roles of family, school, health professionals and health authorities are of utmost importance for the implementation of measures to approach this health problem in a more efficient way. (www.actabiomedica.it)

Key words: self-medication, adolescents, potential risks, dysmenorrhea, health problem

Background and definitions

Self-medication (SM) is widely practiced in both developed and developing countries. The WHO defines SM as the selection and use of medicines by individuals to treat self-recognized illnesses or symptoms (1) and cites SM as a common problem leading to incorrect use of medicine (2). It also includes the use of a wide range of complementary and alternative medicines (CAM), such as herbal medicines (herbs or herbal preparations), nutritional supplements, traditional products, and home remedies (3).

Medicines that require a doctor's prescription are called prescription products (Rx products). Medicines for SM are often called "non-prescription" or "over the counter" (OTC), and are available without a doctor's prescription through pharmacies. In most countries OTC products are also available in supermarkets and other outlets (4,5).

Responsible self-medication requires that: a) medicines used are of proven safety, quality and efficacy; b) medicines used are those indicated for conditions that are self-recognisable and for some chronic or recurrent conditions (following initial medical diagno-

sis). In all cases, such products should be supported by information which describes: i) how to take or use the medicines; ii) the effects and possible side-effects; iii) how the effects of the medicine should be monitored; iv) possible interactions; v) precautions and warnings; vi) the duration of use and vii) when to seek professional advice (5).

SM is normally used for the prevention or treatment of minor ailments or symptoms which do not justify medical consultation. In some chronic or recurring illnesses, after initial diagnosis and prescription, SM is possible with the doctor retaining an advisory role (5-8).

Responsible SM has been defined as the healthcare assumed by the patient where he or she has a greater degree of responsibility in the management of a symptom or illness, using a pharmaceutical product available OTC (4, 5). In this context, pharmacists have an important role to play because they are the most trusted and easily accessible healthcare professionals available to the public as compared to other healthcare professionals. Whenever necessary and appropriate, the pharmacist should refer the patient to a physician (9, 10).

Factors influencing SM include socio-economic status, lifestyle, ready access to drugs, patient satisfaction with the health care provider(s), cost of the drugs, exposure to advertisements, internet access and skill of its use, family influence, educational level, age and gender, pharmacists, previous prescribed drug, or suggestions from an advertisement in newspapers or popular magazines (4, 11-13) Moreover, those with previous experience of using SM and with mild illness are more likely to practice SM (13).

Several studies have reported that SM starts with the onset of adolescence and increases with age (10-12), and is influenced by peers and parents (14-16). Therefore, the issue of SM among adolescents represents an emerging topic in scientific research. The present mini-review aims to describe the prevalence, attitude and practices of SM among adolescents and report how adolescent girls with dysmenorrhea self-manage pain in their daily life.

In the past years, SM has been studied in many countries and several articles have been published on its use in the general population of adolescents, adults, and university students, and in individuals with different health problems. In adolescents the prevalence of SM ranges widely from 2 to 92% (17-21).

A study investigated in 477 students (aged 14-16 years, mean 15.2 years; 53.8% were girls), attending Junior Lyceums in Malta (representing 33.3% of the total adolescents in that age range): (a) the prevalence of self-reported health complaints, (b) the consumption of commonly used medicines, including SM, and (c) the sources of medicines that had been accessed during the preceding 3 months (22).

The most prevalent health complaints experienced by the study population were ear problems, hay fever, cold, cough, headache and skin complaints. One hundred and fifty two girls (59.6% of the total female population) reported menstrual pain. With regard to the overall use of medicines, a total of 428 students (90.3%) had used between 1 to 9 types of medicines including those used for menstrual pain during the preceding 3 months (mean: 2.5 medicines). There was a statistically significant positive correlation between the number of physical health complaints and number of medicines used (r: 0.623, $p<0.001$ for boys and r: 0.573, $p<0.001$ for girls) (22).

The great majority of the students obtained their medicines from a community pharmacy, during the preceding 3 months. However, the source that was mostly accessed by students who had complained of headache and menstrual pain was the home medicine cabinet (53.9% and 60.0% respectively). Antibiotic SM was reported by 9.9% of the students.

In most cases, the participants had obtained medicines with adult guidance. Nevertheless, 104 (24.3%) students had taken at least 1 medicine from friends/young relatives or from the home medicine cabinet without guidance during the same period of time (22).

The prevalence of SM among children aged 0-17 years was studied by Du and Knopf in Germany (23). All cases of last-week medicine use were recorded among 17.450 children, aged 0-17 years.

25.2% of participants had used SM, including drugs acting on the respiratory system (32.1%), alimentary tract and metabolism (21.6%), skin (14.2%), nervous system (11.3%), and homeopathic preparations (8.6%). Overall, girls showed a significantly higher level of self-medication use vs. boys (26.0% *vs.* 24.4%;

p:< 0.05). Among adolescents, aged 11-17 years, boys used significantly more vitamin and mineral supplements than girls, whereas girls used significantly more analgesics (aspirin and paracetamol) than boys. Girls aged 3-13 years used significantly more skin products before puberty than boys, and girls aged 14-17 years used significantly more musculoskeletal system drugs during puberty than boys (23).

However, the study did not separate SM taken by teenagers themselves from those given to them by their parents and did not collect information concerning teenagers sharing medicines with each other, rendering it unfeasible to explore the possible motives of self-medication (23).

Of the 4.294 children and adolescents on SM, 1.309 (30.4%) used prescription medicines concomitantly, and 1.001 (23.6%) used two or more SM simultaneously (ranging from two to seven medicines).The prevalence of multiple medicine use was 5.9% (95% CI 5.3, 6.6) for two or more self-medications, 7.4% for one SM plus at least one prescription medicine and 11.5% for overall (23).

In another recent study (20), of 2.849 self-medicated adolescents, 1.892 adolescents (66.4%) self-medicated one drug, 457 (16%) self-medicated two drugs, 291 (10.2%) self-medicated three drugs, and 209 (7.3%) self-medicated four or more drugs in the past year.

Use of SM was closely associated with the age of adolescents (14 to 17 years), children with poor health status, no immigration background, families with a higher household income and with mothers with a middle to high educational level (23).

The higher use of analgesics by girls than boys (>10 years old) was consistently observed in many other studies (24-28). An international survey of self-reported medicine use among adolescents in 28 countries, including Germany, reported that from 21.1% to 49.9% of boys, and from 28.3% to 65.9% of girls, had used medicines for headache or dysmenorrhea in the previous month (24).

An anonymous self-administered questionnaire was distributed to 1.110 secondary school students in 10 government schools of Kuwait (19). Their mean age was 16.2 years, 601 were males and 509 females. Sixty-five percent of medicines used were for pain relief, 54% for respiratory conditions, 39% for allergic conditions, 37% for dermatological conditions; 23% were nutritional products and vitamins, 21% were gastrointestinal products, 17% were antidandruff products, 15% were hair products, 13% were medicines for migraine and 8% were athlete's foot products. Four hundred and ninety-nine (74%) of the female students indicated that they used medications to manage menstrual discomfort: 224 (45%) used paracetamol, 92 (18%) herbs, 76 (15%) mefenamic acid, 69 (14%) hyoscine preparations, 41 (8%) aspirin, 30 (6%) ibuprofen, while 56 (11%) used other products (19). Few adolescents consulted pharmacists. Female students were more likely to seek advice from parents while male students were more likely to seek advice from a doctor (p = 0.01). SM use tended to increase with age and differed between male and female students.

Three hundred and two (64%) of the male students reported use of SM for muscular pain: 151 (32%) used various creams for rubbing, 140 (30%) paracetamol, 56 (12%) aspirin, 18 (4%) ibuprofen, 16 (3%) diclofenac, and 22 (5%) used other medications (19).

The most common source of general information regarding SM was parents. For the use of SM during acute illness 57% of respondents stated that they would seek advice for medications from a doctor, 36% from their parents, 6% would follow their own instincts while only 1% would seek the advice of a pharmacist. Female students were more likely to seek advice from parents while male students were more likely to seek advice from a doctor (p: 0.01). A total of 701 (64%) stated that they would like to receive more information on the medication they used (19). Patient's education and awareness campaigns were recommended by the authors.

Three systematic reviews of global trends and factors influencing SM practices among adolescents were published between 2010 and 2015 (29-31).

Pfaffenbach et al. (29) screened 403 articles, including 12.013 children and adolescents. The SM frequency varied from 7.0% (24) to 67.7%. The most consumed pharmacological groups were analgesics/anti-inflammatory drugs, followed by antibiotics and estrogens/progestogens.

An additional database search was done by Shehnaz et al. (30) on adolescents aged 13-18 years,

between January 2000 and December 2013. One hundred and sixty-three publications met the inclusion criteria. SM prevalence, in different countries, ranged from 2% to 92%. Headache, allergies, and fever were the most common reported self-medicated health complaints. Female gender, older age, maternal education, and familial practices were associated with increased SM among adolescents (30). The primary sources of drug information, recommendation, and procurement were pharmacists, parents, and friends. High-risk practices, such as diversion of prescription medicines and utilization of previous prescriptions were also reported. Few adverse drug reactions were reported, probably because of lack of awareness about the potential harmful effects of medicines (30).

Gualano et al. (31) selected 15 articles, published from January 1990 until January 2014, involving 143.213 adolescents aged from 13 to 18 years. Overall, 50% of adolescents took drugs without consulting a physician. Only one study reported the possible adverse effects related to the inappropriate use of drugs, which were experienced by 31.1% of the females and 19.6% of the males. Several teenagers, especially girls, aged 11-15 years, admitted to taking different types of drugs, especially painkillers. The major reasons supporting SM were the ease of acquiring, and the affordability of drugs. Furthermore, teenagers did not know how to use drugs and need more information about this topic to prevent side effects (31).

In 2016, a national representative sample of 6,226 students from 99 primary, middle, and high schools in Taiwan completed an online self-administered questionnaire (20). The prevalence of self-medication among the adolescents surveyed was 45.8%. The most common health complaints for SM reported by the participants were cough or cold (75.2%), followed by headache (59.7%), fever (45.8%), stomach disorder (31.6%), intestinal disorder (22.9%), allergy (22.6%), eye disease (14.6%), and dysmenorrhea (17.7%, female). The most frequently used drugs for SM were nonsteroidal anti-inflammatory drugs or pain relievers (31.1%), cold and cough medicines (21.6%), analgesics (19.3%), and antacids (17.3%). The main sources of information for the drugs used for SM were pharmacists (82.9%) and parents (60.2%) (20). Multivariate analysis indicated that adolescents with lower medication knowledge, lower self-esteem, and substance users were more likely to engage in inappropriate SM.

We can conclude that SM is a worldwide phenomenon, the prevalence of which differs depending on the population, the method and the recall period employed for the study. Understanding the interaction between various factors promoting SM can be helpful in promoting strategies to reduce drug-related health risks among adolescents. Parents are the most important source of information for the use of SM. The prevalence of SM was higher in female adolescents in most countries. Therefore, information from professionals should be made available and addressed to adolescents and parents with the aim of creating awareness in the general population on the potential risks of using drugs without proper information and consultation.

Inappropriate practice of self-medication and potential risks

Although benefits are linked to appropriate SM, the increased access to medication for the treatment of "minor health conditions" raises the possibility of non-responsible SM. This includes: incorrect self-diagnosis, delays in seeking medical advice when needed, infrequent but severe adverse reactions, dangerous drug interactions, incorrect manner of administration, improper dosage, wrong choice of therapy, lack of awareness of warnings and precautions, inadequate storage conditions and ignorance of the recommended shelf-life, polypharmacy, and drug abuse (20, 32). Furthermore, many diseases have similar symptoms, and a person relying on previous experience to use SM may be exposed to the dangers of misdiagnosis and consequently improper treatment (13).

Low health literacy was also associated with inappropriate SM (33), less adherence to self-care regimens (34), more medication errors (35), and higher risk of hospitalization (36). In addition, a study showed that frequent alcohol drinking was a risk factor for increased SM among adolescents (37).

The potential benefits and risks of SM at the individual and community level are reported in Table 1.

Table 1. Potential benefits and risks of self-medication at individual and community level (From Ref. 4)

Potential benefits at individual level	**Potential risks at individual level**
• Active role of adolescents in health care • Self-reliance in preventing or relieving minor symptoms or conditions • Convenience • Economy, since medical consultations will be reduced or avoided.	• Incorrect self-diagnosis • Failure to seek appropriate medical advice promptly • Incorrect choice of therapy • Failure to recognize special pharmacological risks • Rare but severe adverse effects • Failure to recognize or self-diagnosis of contraindications, interactions, warnings and precautions • Failure to recognize that the same active substance is already being taken under a different name • Failure to report current self-medication to the prescribing physician (double medication/ harmful interaction) • Failure to recognize or report adverse drug reactions • Incorrect route of administration • Inadequate or excessive dosage • Excessively prolonged use • Risk of dependence and abuse • Food and drug interaction • Storage in incorrect conditions or beyond the recommended shelf life • Drug-to-drug interactions
Potential benefits at community level	**Potential risks at community level**
• Alleviate medical resources from being wasted on minor conditions • Lowering the costs of community funded health care programs • Reducing absenteeism from school due to minor symptoms • Increasing the availability of health care to populations living in rural or remote areas.	Improper self-medication could result in an increase in drug induced disease and in wasteful public expenditure.

Adverse drug reactions

Despite numerous studies on adverse drug reactions (ADRs) related to SM in hospitalized patients (38,39), there are few available data related to the rate and severity of ADRs in SM.

Goldsworthy and Mayhorn (40) interviewed 594 adolescents throughout the United States. One in five reported sharing prescription medication. Of these, less than half received instructions, many delayed professional care, few informed providers, and a third reported experiencing side effects.

A self-completion questionnaire was designed and used by Westerlund et al. (41) in 245 Swedish students (138 females and 107 males; median age of 17 years) in order to identify their experience about drug-related problems (DRPs). DRPs had been experienced by 31.1% of females and 19.6% of males. The most common DRP was therapy failure in 46.5% of the girls and 38.1% of the boys. Physicians solved the problems in 41.4% of the cases.

Among people who practice SM, adolescents were at higher risk for inappropriate use, particularly with antibiotics (42,43). Misuse and overuse of antibiotics lead to numerous individual and societal problems, among which antimicrobial resistance (AMR) that is currently a major worldwide concern (42, 43). Therefore, WHO has stressed the need to raise awareness about the proper use of antibiotics and the threat of AMR in the general population (44)

A large European survey has shown that adolescents and young adults (aged 15-24 years) are the highest users of antibiotics (43) and are more likely than other age groups to take them for upper respiratory tract infections (URTIs). Penicillins are the antibiotics most used for upper respiratory tract infections, supposedly to provide a quick relief from illness. Parents have an advisory role, acting to limit or encourage use (45).

Most adolescents perceive that antibiotics are specifically for bacterial infections and some feel they are no different from other medications such as painkillers. There was some misunderstanding about the difference between viral and bacterial infections. In general, the majority of adolescents have poor understanding of AMR. Comparison with data from other countries confirms similar findings, but in countries where antibiotics are available "over the counter" without prescription the reported overuse of antibiotics is higher (43).

Drug-to-drug interaction is another potential danger associated with SM. In a study among U.S. adolescents, most girls used one analgesic or anti-inflammatory drug, whereas one-third of them reported using two to three analgesics for dysmenorrhea (not concurrently) (46). Nearly all used at least one medication, 31% reported using two, and 15% used three medications (not concurrently). In another population, SM practices were also inappropriate, regarding appropriate drug choice, therapeutic doses, and associated side effects, in a substantial proportion of young adult women with primary dysmenorrhea (47).

In summary, studies suggest that SM is influenced by many factors such as education, family, society, law, availability of drugs, exposure to advertisements and others. Parents and adolescents should be aware of the principles of proper/improper use of medicines, their potential side effects, and the strict regulations on non-prescription antibiotics and painkillers. An important aspect to be considered for the use of anti-inflammatory drugs is related to their safety, especially for long-term use.

The paradigm of self-medication among adolescent girls with dysmenorrhea

Despite a plethora of worldwide studies on dysmenorrhea, there are few studies on self-care strategies for dysmenorrhea (DS) in young girls. DS is commonly categorized into two types; primary and secondary. Primary dysmenorrhea (PD) is defined as painful menses with cramping sensation in the lower abdomen that is often accompanied by other symptoms, such as headache, nausea, vomiting, diarrhea, backache, and leg pain. All these symptoms occur just before or during the menses in women with normal pelvic anatomy. Several studies suggest that severe menstrual pain is associated with absenteeism from school or work and limitation of other daily activities. One-third to one-half of females with PD miss school or work at least once per cycle, and more frequently in 5% to 14% of them (48). Dysmenorrhea may be also secondary to pelvic organ pathology such as endometriosis, pelvic inflammatory disease, IUDs, ovarian cysts, adenomyosis, uterine polyps.

Wong and Khoo (49) performed a cross-sectional study in 1.092 Asian girls from 15 public secondary schools. 76.1% of the participants believed that dysmenorrhea was a normal part of the female menstrual cycle and only 14.8% sought medical treatment. Similar results were reported in female nursing school students in Taiwan (50).

We performed a systematic search, using PubMed and Google Scholar, in order to identify publications on the self-care strategies of adolescents for managing DS. The following keywords were included: "self medication, adolescent, self prescription, over the counter preparations, self care, self administration and dysmenorrhea". All papers written in English, French, Portuguese, Spanish and Italian were considered, resulting in review of 226 papers.

Davis et al. (51) collected via interview the self-treatment patterns among 76 adolescents with moderate or severe DS. The mean age was 16.8 years (range 11-19). Dysmenorrhea was moderate in 42% and severe in 58%. Subjects reported numerous coping strategies for DS. These included discussing their pain with someone, including their mother (84%), friend (67%), doctor (37%) or nurse (22%). Those with severe DS were not more likely to seek medical care than those with moderate pain (p: 0.7). Nearly all subjects used non-pharmacological remedies, 84% stayed in bed or slept, 75% took a hot bath, 50% used a heating pad, 47% watched television or tried to distract themselves, and 30% did exercise.

Ninety percent reported using OTC medication, and 21% reported using prescription medication. The most common OTC medications used were ibuprofen (54%), acetaminophen (41%), Midol (28%), and naproxen (17%). Thirty-nine percent of subjects reported using two types of OTC medication, and 16% used three types (but not simultaneously). Most subjects

were doubtful of the dose used (53%) and took fewer than the recommended number of pills for pain.

A total of 1.231 college girls with PD, aged between 16 and 23 years, in Changsha (China), completed a questionnaire, including their sociodemographic characteristics, anthropometric measurements and maternal history of DS (51). Most girls with PD changed their lifestyles to avoid inducing or aggravating menstrual pain and other discomfort. For example, 94.6% of the girls with PD (n:1.165) reported that they would reduce physical activity by avoiding heavy personal cleaning or housework or increasing time for rest. Additionally, 56.5% (n: 695) reported that they would use complementary therapies, mainly heat therapy (51). Friends or classmates and mothers were the most important persons to whom girls turned to for answers regarding methods for dealing with PD (52).

The results of additional studies, collected from 2009 to 2019 in adolescents and young adults, are summarized in Table 2. The limitation of these studies was that they did not distinguish between PD and secondary dysmenorrhea.

In summary, treatment for DS varies across different populations worldwide. Despite its frequency and severity, most adolescents do not seek medical treatment for dysmenorrhea or consult health care professionals. They consider painful periods as normal

Table 2. Persons consulted and self-care management of dysmenorrhea in adolescents living in different countries

Study	Country	Design	Sample size	Person consulted (%)	Self-care (%)
Ortiz MI et al. Int J Gynecol Obstet. 2009; 107: 240-243.	Mexico	Q	1.152 high school students. Age: NA	NR	**Pharmacological:** Combination paracetamol, pamabrom and pyrilamine maleate; NSAID alone or with butylhioscine.
Nwankwo et al. J Pediatr Adolesc Gynecol.2010; 23:358-63.	Nigeria	CSS	Postmenarcheal adolescent school girls, aged 10-19 yrs.	16% of adolescent girls sought medical advice regarding DS	**NA**
Wong LP et al. Aust J Rural Health 2011; 19:218 -223.	Malaysia	CSS	1.295 adolescent girls, aged 13-19 yrs.	Mothers: 62.3% Peers: 52.9%.	**Pharmacological:** The majority were worried about dependence and side effects. **Non-pharmacological**: Heat therapy and traditional Chinese medicine.
Chia CF et al. Hong Kong Med J 2013; 19:222-228	Hong Kong	CSS	128 medical and 112 non-medical students. Mean age 20.1± 1.4 yrs.	Medical advice: 6%.	**Pharmacological:** Paracetamol (56 %) NSAID (100 %) TCM (93%) **Non-pharmacological:** Warm beverage (50 %) Sleeping (64 %) Postural adjustments (58 %) Warm water bag (77 %) Exercise (51%) Chocolate (47%) Dietary/nutritional supplements (92 %)
Wijesiri HS et al. Nurs Health Sci. 2013; 15: 58-64	Sri Lanka	CSS	168 students, from 17 to 18 yrs of age, suffering from DS.	Mother: 92.8 % Friends: 20.5 % Mass media: 8.7 %, teachers: 5.1 %, sisters: 5.9 %, father: 4.1 %, healthcare providers 0.8 %.	**Pharmacological:** Medications: 55% **Non-pharmacological:** Rest: 64% Hot fomentation:20% Exercise: 5% Yoga: 3%

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Table 2 *(continued)***.** Persons consulted and self-care management of dysmenorrhea in adolescents living in different countries

Study	Country	Design	Sample size	Person consulted (%)	Self-care (%)
Farotimi AA et al. J Basic Clin Reprod Sci 2015; 4: 33-38.	Nigeria	Q	315 students, aged 18-23 yrs; mean age of 19.1 ± 0.95 yrs.	Friends: 65.2% Mother/Sisters:4% Doctors: 23.0% Healthcare provider: 7.9 % Teacher: 4.4%	**Non-pharmacological:** Herbal medicine (69.1%) and home remedies, such as cinnamon and anise tea.
Aktaş D. Pain Manag Nurs. 2015; 16:534-543.	Turkey		200 female students; mean age was 20.85 ± 2.15 yrs.	1/4 of the students with DS consulted a physician.	**Pharmacological:** Analgesics (69%) **Non-pharmacological:** Heat application (56.5%) and rest (71.4%).
Omidvar S et al. Glob J Health Sci. 2016 Aug 1;8 (8):53632. doi: 10.5539/ gjhs. v8n8p135.	South India	CSS	1000 healthy females aged 11-28 yrs; 47.8% < 18 yrs.	Only 14.2% had sought medical advice	**Pharmacological:** In ~ 25.5% of subjects **Non-pharmacological:** In 83.2% of subjects.
Subasinghe AK et al. Aust Fam Physician. 2016;45:829-834.	Australia	Q	247 females, aged 16–25 yrs.	Approximately 86% had information from internet, social media, magazines, family members and friends	**Pharmacological:** Paracetamol, aspirin, mefenamic acid and ibuprofen (58%). **Non-pharmacological:** Heat packs: 54% Hot baths: 37% Meditation: 7% Exercise: 2%.
Kamel DM et al. J Pain Res. 2017:10; 1079-1085	Egypt	CSS	269 female college students. Mean age 20.4 ±1.7 yrs.	Most students (91.2%) did not seek medical consultation for DS.	**Pharmacological:** 62.4 % used analgesics. **Non-pharmacological:** Drink hot liquids:56.6% Rest:50% Hot application:34.1% Massage: 15.0% Exercise: 14.2% Herbs: 9.7% None:15.9%.
Oksuz E et al. Konuralp Tıp Dergisi 2017;9:37-45	Turkey	CSS	190 female university students; mean age 20 yrs.	Sources of information were: 59.0% mother: 26.8%; a health professional; 5.7%; a family member other than the mother: 3.5%; friends and 5.0% teacher.	**Pharmacological:** In 44.7% of females, the analgesic used was proposed by a physician and in 29.8% by someone in the family. 53.8% female students were using more than one analgesic in a day: the time between two analgesics was 1-2 hrs.
Saeed AA. Iraq. Med J Babylon 2018;15:150-154.	Iraq	CSS	300 adolescent students with DS, aged between 10 and 21 yrs.	NR	**Pharmacological:** Medications:10.6% **Non-pharmacological:** Herbal treatment:9.3%, sweet or spicy foods (48,0%), chocolate and massages (17.2%, for each), Sleeping: 17%

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Table 2 *(continued)***.** Persons consulted and self-care management of dysmenorrhea in adolescents living in different countries

Study	Country	Design	Sample size	Person consulted (%)	Self-care (%)
Acheampong K et al. Obstet Gynecol Int. 2019 May 20; 2019:5834159. doi: 10. 1155/ 2019/5834159	Ghana	CSS	760 healthy adolescents aged 12-19 yrs.	56.6% ignored their menstrual pain. Few (19.4%) of them consulted a physician.	**Pharmacological:** SM: 34.6%, **Non-pharmacological:** Relaxation: 25.7%, hot application:11.4%, herbs: 6.7%, physical exercise:14.9%.
Chen L et al. BMJ Open 2019;9:e026813. doi:10.1136/ bmjopen- 2018-026813	China	CSS	2.555 college girls, aged between 16 and 23 yrs.	Friends or classmates and mothers: 89.3% Medical advice: 27.4%	**Non-pharmacological:** Lifestyle changes: 56.5% SM: 34.8%, with 15.6% taking Western medicine, 8.6% taking traditional Chinese medicine and 10.6% taking both.
Kizilirmak A et al. Med Sci, 2019; doi: 10. 5455/ medscience. 2018.07.8937	Turkey	Q	3.526 girl students, aged 20.5 ± 1.7 yrs.	Friends (13.8 %) Doctor (38.7 %) Midwife/Nurse (3.1 %) Family members (22.3 %) Pharmacy (22.0 %) Blogs or internet) (1.9 %)	**Pharmacological:** 63.2% used analgesic: Paracetamol (41.4 %), NSAID (64.2 %) Relaxant (2.0%) Antispasmodic (5.6 %).
De Sanctis V. 2019; personal observations.	Italy	CSS	74 adolescents, aged 16.2 ± 2.1 yrs. with moderate or severe DS were interviewed	Family members (60.8 %); a health professional:13.5%; internet, social media, friends, magazines (25.6%)	**Pharmacological:** Paracetamol: 35.1% NSAIDs:39.1% Antispasmodic:4.0%. **Non-pharmacological:** Rest/sleeping:14.8% Heat application:6.7%.

Legend: **Q:** Questionnaire**; CSS:** Cross-sectional survey; **DS:** dysmenorrhea; **NSAID:** non-steroidal anti-inflammatory drugs; **TCM:** traditional Chinese medicine

conditions that they can handle using different self-care methods (53). This may be also related to the fact that they have more opportunities to obtain "medical" advice from their parents, friends, pharmacist, newspapers or popular magazines and internet. However, few studies have been performed on SM of DS in adolescents living in developed countries. It would be advisable to give more attention to this public health concern and to promote initiatives in order to promote citizen awareness about the risks related to the consumption of drugs without medical consultation, and to educate adolescents and young women to seek professional healthcare when they experience moderate-severe DS.

Conclusions

SM is an important economic, social and health issue throughout the world. Numerous studies have been conducted in many countries to investigate SM practice among different groups of the population including adolescents. For minor illnesses and common symptoms, SM often provides a cheap, rapid, and convenient solution, which considerably lessens the burden on the health care system of any country. The pattern of self-treatment varies in different communities and is affected by several factors such as: age, sex, income, expense, self-care orientation, education level, medical knowledge, satisfaction, and perception of disease. The negative aspect of SM is that most people are not aware of the side effects and proper dosage of the drug to be used, or of drug interactions. The World Health Organization found that SM in individuals with lower medication knowledge may result in several potential risks.

The high prevalence rate of self-medication with analgesics is consistent with our observation of "head-

aches", fever and DS as the most common indications behind SM.

Strategies to control and minimize the risks of SM should involve monitoring systems, promotion of education, spread of sound information, and encouraging a partnership between patients, physicians and pharmacists (54,55). Appropriate counselling and management should be available to female students to help them cope with the challenges of DS. The reluctance of girls to seek medical advice suggests that girls have incomplete or incorrect knowledge about DS and consequently suffer unnecessary pain. Health education about pain due to potential secondary dysmenorrhea is also urgently suggested (56).

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