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The relationship between abdominal fat, glucose-regulated protein 78, and endometrial cancer

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Summary. *Background and aim:* The adipocyte is the central element that integrates multiple metabolic and endocrine signals. This cell is the source for a multitude of bioactive peptides that play an essential role in endometrial cancer pathogenesis. The association of obesity with endometrial cancer is supported by Glucose-regulated protein 78 (GRP78), a marker of the stress endoplasmic reticulum (ER). GRP78 has an important anti-apoptotic role. Recent studies have demonstrated that GRP 78 plays an important role in the development, progression and tumor chemoresistance. The study aimed to identify a correlation between abdominal obesity, plasma GRP 78 level an d endometrial cancer. *Methods:* Two groups of patients were included in the study: group I – 44 patients diagnosed with endometrial cancer, group II – 44 patients without gynecological pathology or inflammatory disorders. After the performance of clinical examination and anthropometric measurements, abdominal fat was determined by dual X-ray absorptiometry and plasma GRP 78 level was measured. *Results:* Plasmatic level of GRP78 was significant higher in patients with endometrial cancer compared to the control group. Abdominal fat is in a positive linear correlation with the plasma GRP78 level (p<0.0001). *Conclusions:* The measurement GRP78 level associated with the determination of abdominal fat can be an useful predictor factor for endometrial cancer.

Key words: GRP78, abdominal fat, endometrial cancer

«Il Rapporto tra grasso addominale, proteina regolata dal glucosio 78 ed il cancro dell'endometrio»

Riassunto. *Contesto:* L'adipocita rappresenta l'elemento centrale che integra molteplici segnali metabolici ed endocrini. Questa cellula è la fonte di una moltitudine di peptidi bioattivi che hanno un ruolo essenziale nella patogenesi del cancro dell'endometrio. L'associazione tra obesità e cancro dell'endometrio è dimostrata dalla proteina regolata dal glucosio 78 (GRP78), un marker di stress del reticolo endoplasmatico (RE). La GRP78 ha un importante ruolo anti-apoptotico. Gli studi più recenti hanno dimostrato che la GRP78 ha un ruolo importante nello sviluppo, la progressione e la chemioresistenza del tumore. Lo studio si propone di identificare una correlazione tra l'obesità addominale, il livello plasmatico della GRP78 ed il cancro dell'endometrio. *Materiali e metodi:* Lo studio comprende due gruppi di pazienti: il primo gruppo composto da 44 pazienti con la diagnosi di cancro dell'endometrio; il secondo gruppo da 44 pazienti senza patologia ginecologica o disturbi infiammatori. Dopo aver effettuato l'esame clinico e le misure antropometriche, il grasso addominale è stato determinato attraverso assorbimetria a raggi X a doppia energia ed è stato misurato il livello plasmatico della GRP78. *Risultati:* Il livello plasmatico della GRP78 era significativamente più alto nelle pazienti con diagnosi di cancro dell'endometrio rispetto alle altre pazienti che facevano parte del gruppo di controllo. Il grasso

addominale rappresenta una correlazione lineare positiva con il livello plasmatico della GRP78 (p<0.0001). *Conclusioni:* La misurazione del livello della GRP78 in associazione con la determinazione del grasso addominale può costituire un fattore predittivo utile nel cancro dell'endometrio.

Parole chiave: GRP78, grasso addominale, cancro dell'endometrio

Introduction

Endometrial cancer (EC) is the most common malignancy within the female reproductive system (37.7%). The incidence increases with age. Frequently this type of cancer is diagnosed in peri- and post-menopausal women. 60-70% of cancers occur in women over 60 years of age, and less than 5% in women below 40 years of age (1). The incidence of EC is 10 times higher in industrialized Western European countries than countries of Asia and Africa (2). In economically developed countries, endometrial cancer is associated with obesity in a proportion of 40% (3).

There are different risk factor associations for endometrial carcinomas, supporting the etiologic heterogeneity of these tumors. Factors that increase the risk of developing endometrial cancer include early menarche, late menopause, age, obesity, diabetes, menstrual disorders, anovulatory cycles, polycystic ovary syndrome, childlessness, hormone replacement therapy and estrogen-secreting tumors (4).

The hypothesis of the alteration of the estrogen-progesterone balance is used to support the relationship between obesity, steroid hormones and the risk of EC, but it cannot explain the effects on the entire population, given that not all obese women develop endometrial abnormalities. The identification of biological markers that indicate an increased risk for the development or recurrence of EC in obese women might be useful for decreasing EC mortality and morbidity.

The strong association between obesity and endometrial cancer may be due to the development of endoplasmic reticulum (ER) stress in adipocytes. Endoplasmic reticulum stress is an adaptive reaction, which normally occurs in any cell that is required to process more molecules than usual per time unit. In this case, there is a risk that part of the incompletely processed molecules (post-translational molecular folding, twisting, packing) might generate an adaptive reaction in ER that consists of the occurrence of molecular conformational defects, which might prevent traffic through this structure (5).

Glucose-regulated protein 78 (GRP78) is a ubiquitously expressed protein in normal cells and a marker of ER. It belongs to the heat shock protein family and is induced by a variety of stress conditions (6). The amount of expressed GRP78 is directly correlated with tumor invasiveness, and can be used as a biomarker for cancer (7). GRP78 supports cell survival, having anti-apoptotic properties (8). GRP78 levels in visceral adipocytes are correlated with the stage of EC, but also with the survival of these patients, and might be clinically useful as a predictor for endometrial cancer (9).

The study aimed to identify a correlation between abdominal obesity, GRP78 levels, and endometrial cancer.

Materials and method

The study is a case-control analysis that includes 2 groups of patients: group I – 44 patients diagnosed with endometrial cancer, group II – 44 patients without gynecological pathology or inflammatory disorders.

The diagnosis of endometrial cancer was made after histopathological examination that analyzed the tissue material obtained following endometrial biopsy.

The anthropometric measurements performed were: body mass index (BMI), calculated by the formula BMI = weight (kg)/[height (m)]^2, and abdominal circumference (AC), measured in orthostatism, at umbilical level. The height of the subjects was accurately measured (error less than 1 mm), by the stretching procedure for height measurement of the Society for the Development of Kinanthropometry, using an anthropometer fixed to the wall (222 model; Seca GMBH, Hamburg, Germany). Body mass was determined with

an accepted error of 20 g using a calibrated electronic scale (FW-150K model; A&D Mercury, Thebarton, Australia).

These patients underwent Dual X Ray Absorptiometry (DXA) examination with a GE Prodigy Lunar device, which measured the android and gynoid adipose tissue content. Abdominal adipose tissue was assessed by total body scans. The "abdominal" region was defined using the software provided by the manufacturer, with the upper limit at the point where the lower 1/5 joins the upper 4/5 of the pelvis-to-chin distance, and the lower limit at the upper part of the greater trochanters.

From each subject included in the study, 6 ml fasting blood was taken by venous puncture and collected in test tubes without anticoagulant for the determination of plasma GRP78 levels. The serum obtained by centrifugation was divided and stored in 600 μl freezing tubes at a temperature of -30^0 C until the tests were performed, in order to avoid repeated freezing-thawing cycles. The serum GRP78 concentration was determined by the sandwich ELISA technique using Human GRP78 Immunoassay MBS031039 (R&D Systems USA). The sensitivity of this kit is 10 μg/ml. The detection rate of this kit is 50 μg/ml. No significant cross reactivity or interference between human GRP78 and analogues was found.

The informed consent of all patients was obtained. The study was conducted under the tenets of the Helsinki declaration.

All parameters were included in the study database. Normal distribution was tested by the Kolmogorov-Smirnov test. Normally distributed variables are presented as means±standard deviation; non-normally distributed as medians (interquartile range). For comparison of two means the t-test or Mann-Whitney test was used. For relationship analysis between two variables, Pearson's or Spearman's correlation coefficient (r) was used. To control for age, partial correlation and multivariate linear regression using the stepwise method was used. The cut-off was taken as the value which maximizes the Youden index using ROC (receiver operating characteristics) curve analysis. $p<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 15.0.

Results

The characteristics of the patients included in the study are described in Table 1.

According to Table 1, patients with endometrial cancer were older, had an early menarche and late menopause. These patients had also a statistically significant higher abdominal circumference and increased abdominal fat.

Plasma level of GRP78 was observed to be significantly higher in patients with endometrial cancer than in non-diseased patients.

The correlations between GRP78 levels and patients' main characteristics were also tested. GRP78 values correlated inversely with age at menarche appearance and patient's age, time of onset of menopause, abdominal circumference and abdominal fat. In order to avoid co-founders, we performed age-controlled correlations and these maintained a similar

Table 1. Characteristics of the patients included in the study.

Characteristics of patients	Control (44 patients)		Cases (n=44 patients)		p
	Mean ± SD	95% CI	Mean ± SD	95% CI	
Age	55.11±8.47	52.54-57.69	71.84±5.69	70.11-73.57	<0.0001
Menarche	13.02±1.09	12.69-13.35	12.25±1.16	11.90-12.60	0.002
Menopause	46.00±3.03	45.08-46.92	51.95±3.38	50.93-52.98	Ns
Weight(kg)	85.61±8.35	83.08-88.15	86.89±8.37	84.34-89.43	Ns
BMI	31.23±2.88	30.35-32.10	31.89±2.93	31.00-32.78	Ns
AC(cm)	90.86±12.75	86.99-96.74	104.05±11.30	100.61-107.48	<0.0001
% Abdominal fat	18172.48±3008.22	17257.89-19087.06	22049.07±2148.03	21396.01-22702.13	<0.0001
GRP78	0.379±0.188	0.322-0.436	1.138±0.095	1.110-1.167	<0.0001

Note: 95% = 95% Confidence Interval (CI) for Mean; SD = Standard Deviation; NS = Not significant

Table 2. Correlations between GRP78 and patient characteristics.

	GRP78 uncontrolled correlations	Age controlled GRP78 correlations
Age		
r	0.73	
p	<0.0001	
Menarche		
r	-0.30	-0.17
p	0.005	0.11
Menopause		
r	0.63	0.47
p	<0.0001	<0.0001
Weight(kg)		
r	0.07	0.01
p	0.51	0.89
BMI		
r	0.10	-0.02
p	0.33	0.84
AC(cm)		
r	0.42	0.23
p	<0.0001	0.02
Abdominal fat (g)		
r	0.57	0.40
p	<0.0001	<0.0001

r = Pearson correlation coefficient; p = value

trend. GRP78 correlated positively with menopause, abdominal circumference and abdominal fat.

According to Table 2, patients reaching menopause at an older age, with high abdominal circumference and increased abdominal fat will have higher GRP78 serum levels.

The positive linear correlation between abdominal fat and plasma GRP78 level can also be followed in figure 1.

In order to establish the influence of the other variables on GRP78, *stepwise* multivariate linear regression analysis was performed. The following variables were entered in regression: age, BMI, abdominal circumference, menarche, menopause, abdominal fat (Table 3).

According to Table 3, age, menopause and abdominal fat proved to be independent predictors of high GRP78 serum levels, directly influencing its variability.

Discussion

Overweight and obesity are associated with increased morbidity and mortality. Morbid obesity, defined as having a body mass index ≥40 kg/m^2, increased by 70% between 2000 and 2010 and is more common among women (10). The risk of mortality of women with endometrial cancer is 6 times greater in the morbidly obese population than in those with healthy BMI, twice as high as the risk seen for those who are obese (defined as BMI = 30-39.9 kg/m^2) (11). Obesity

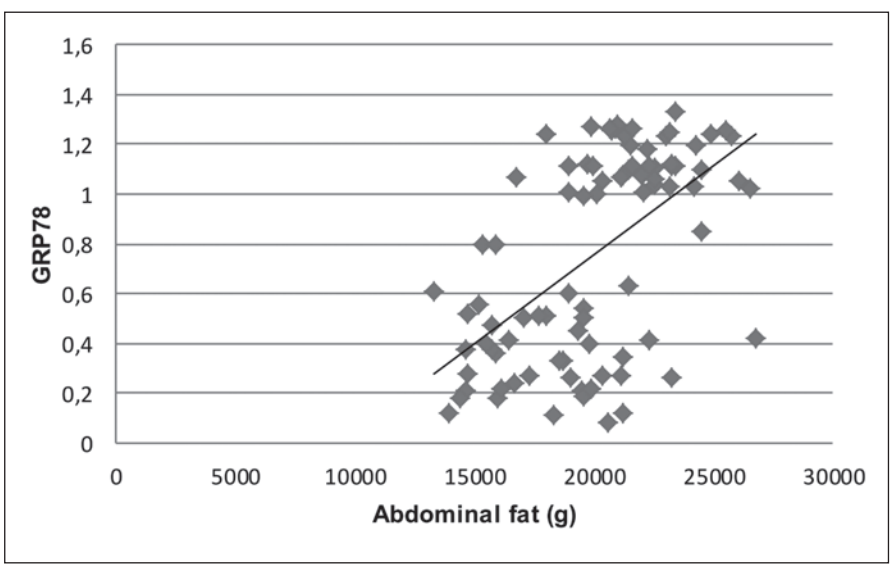

Figure 1. Correlation between GRP78 and abdominal fat.

Table 3. Multiple linear regression analysis of parameters associated with GRP78 levels (Dependent Variable: GRP78).

Coefficients(a)	Unstandardized Coefficients		p value	95% Confidence Interval for B	
	B	Std. Error		Lower Bound	Upper Bound
(Constant)	-1.92	0.35	<0.0001	-2.631	-1.226
Age	0.01	0.002	<0.0001	0.008	0.019
Menopause	0.02	0.006	<0.0001	0.013	0.039
Abdominal fat (g)	0.00003	0.00001	0.001	0.00001	0.00005

has been linked to several pathological conditions including impaired glucose level and lipid metabolism, insulin resistance, increased predisposition to cancers of the colon, breast and prostate (12).

DXA for determining the composition of the body as well as composition by regions is increasingly being used. When using DXA, only two body compartments can be measured concomitantly (13). Initially, DXA allows the division of the body into two compartments: the bone mineral and the soft tissue compartment. Soft tissues can be divided in turn into two molecular compartments: fat and fat-free tissue (14).

DXA has been considered the gold standard for body composition measurements in obese people (15). Anthropometric methods are not reliable methods in obese patients and single frequency-bioelectrical impedance analysis, foot-to-foot impedance meters and upper body fat analyzers have also been reported to be inaccurate in subjects with a BMI > 34 kg/m^2 (16).

The effect of adipose tissue distribution on EC is far from being clear. Adipose tissue is metabolically heterogeneous, with variations between visceral and subcutaneous adiposity regarding adipokine synthesis and steroid hormone metabolism regulation. Android fat distribution (also termed abdominal, central, visceral or upper body fat distribution) leads to an increase in the risk of chronic disorders such as cardiovascular disease or type 2 diabetes mellitus, while a larger hip and thigh circumference which is present in gynoid fat distribution is associated with a reduction in the risk of metabolic disorders.

In both this study and literature studies, abdominal circumference is an effective predictor of abdominal fat tissue. In 2001, the National Cholesterol Educational Program included AC as a risk factor for metabolic syndrome. AC might be a better predictor of obesity risk in the case of the Asian population because Asian subjects tend to have a higher body fat and visceral fat percentage than Caucasians or Afro-Americans, within the same BMI range of values (17). However, the correlations between AC and visceral adipose tissue may vary between 0.4 and 0.9, depending on sex, age and the severity of the disease (18).

Anthropometric measurements such as AC and BMI are generally used as an indirect measure of abdominal adipose tissue in clinical practice, as well as for large epidemiological studies in which DXA is not available. Although much more rigorous, DXA requires expensive equipment, a trained technician, and exposes the participants to radiation.

The effects of adiposity on the relationship between fat distribution and EC have not been studied on a wide scale. Given that increased obesity is generally associated with increased abdominal obesity, BMI can be an external variable in the relationship between android obesity and EC. Both overall adiposity and body fat distribution is strongly associated with endometrial cancer risk, the latter being a stronger disease predictor. The association between central obesity and endometrial cancer was more pronounced among younger women and was seen for both normal-weight and obese women (19).

According to our study, DXA is an effective method for the determination of abdominal adipose tissue. The value of DXA is clearly superior to anthropometric indices, particularly when it concerns the evaluation of abdominal adipose mass, because it allows one to assess the adipose tissue content in various body subregions (20).

Obesity is a risk factor for endometrial cancer (21). However, the prognostic utility of obesity for the development of endometrial cancer is still being assessed. The association of obesity with endometrial cancer represents a double molecular injury to the

endoplasmic reticulum; thus, the triggered unfolded protein response is present at both tumoral and adipocyte level. This double injury is also determined by the association of endometrial cancer with inflammation, evidenced by the presence of inflammatory factors in blood circulation (adiponectin, leptin, resistin, omentin, TNF-α, IL-6, etc.). Despite the strong association between obesity and endometrial cancer, it is not clear whether ER stress develops in the adipocytes of these patients; it is on this idea that the latest studies regarding the elaboration of a new non-invasive method for the diagnosis of endometrial cancer are based. The results of these studies have demonstrated that GRP78 levels in visceral adipocytes are correlated with the disease stage and patient survival, and might be clinically useful as a predictor of endometrial cancer (22).

Insulin and insulin-like growth factor-1 (IGF-1) stimulate protein synthesis, anti-apoptotic proliferation and signaling through the induction of mitogen-activated protein kinase (MAPK). GRP78 expression is a target downstream of insulin, recent evidence suggesting that GRP78 and the global protein balance of the endoplasmic reticulum can regulate the insulin sensitivity of the body and can protect cells during acute stress. Obesity and type 2 diabetes mellitus are metabolic disorders characterized by insulin resistance and hepatic steatosis. The presence of ER stress in the context of metabolic syndrome has been documented (23, 24), and the presence of chaperones might play a key role in the regulation of insulin sensitivity and glucose homeostasis.

GRP78 mRNA was diminished in the adipose tissue of patients with gastric bypass after weight loss, which means that the relationship between obesity-related ER stress and metabolic dysfunction is present in humans (25).

GRP78 overexpression is widely reported in cancer cell lines, associating with aggressive growth and invasive properties (26). In sustaining ER protein folding capacity and maintaining ER stress sensors and ER associated pro-apoptotic machineries in their inactive state, GRP78 regulates the balance between cancer cell viability and apoptosis (27, 28). GRP78 might also promote cell proliferation from the cell surface. ER stress or ectopic expression of GRP78 leads to localization of a subfraction of GRP78 on the cell surface (29). It also regulates inflammation and immunity through multiple mechanisms (30). As a major ER chaperone, GRP78 facilitates the processing and secretion of cytokines and chemokines (31). This study supports the idea that abdominal obesity is directly correlated with plasma GRP78 levels, as well as with endometrial cancer, suggesting that GRP78 is involved in the pathogenesis of EC.

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