Original article

Iron status in healthy subjects living in Jordan

Ala A. Qatatsheh[1], Ibrahim R. Dabbour[2], Abdallah S. Al-rethaia[3], Murad A. Al-Holy[4], Sharaf S. Omar[5]

[1] Department of Nursing, Princess Aisha Bint Al-Hussein Faculty of Nursing, Al-Hussein bin Talal University, Ma'an, Jordan and Department of Clinical Nutrition, Faculty of Applied Health Sciences, Qassim University, Arrass, Saudi Arabia - E-mail: seif1972@yahoo.com; [2]Department of Nutrition and Food Technology, Faculty of Agriculture, Mutah University, Karak, Jordan; [3]Department of Health Sciences Preparation, Faculty of Applied Health Sciences, Qassim University, Arrass, Saudi Arabia; [4]Department of Clinical Nutrition and Dietetics, Faculty of Allied Health Science, The Hashemite University, Zarqa, Jordan; [5]Department of Nutrition and food Processing, Faculty of Agricultural Technology, Al-Balqa Applied University, Salt, Amman, Jordan

Summary. Iron status was determined in 240 subjects who referred for routine check up in Zarqa governorate hospital located in Jordan. Prevalence of ferritin deficiency (<16µg/l) was 3.4% (n=8) in males and 24.1% (n=58) in females of the studied population (P<0.05), while ferritin concentration >700µg/l was not observed. Prevalence of serum iron deficiency (<60 ng/mL) was 5.4% (n=13) in males and 18.8% (n=45) in females (P=0.01). Mean serum iron was 52.1±69.07 µg/l in females and 117.7±107.9 µg/l in males over 25 year old age (P=0.03). However, estimated mean serum concentration of ferritin was not affected by age and gender. These results revealed that Jordanian males eat higher dietary intake of iron than females, which is adequate to maintain adequate iron status with a low prevalence of iron deficiency.

Key words: serum iron, serum ferritin, deficiency

Introduction

Iron deficiency (ID) remains widespread in the world, its overall prevalence is near to 10% and 50% in developed and developing countries respectively (1,2). ID has negative consequences in all age groups, if it is not treated in the early stages of the disorder it will develop iron deficiency anemia (IDA) (3). It is well known that the main causes of ID include nutritional deficiency, absorption problems, high iron demands and an excessive blood loss, inability to form hemoglobin in the absence of other necessary factors such as vitamin B12, lack of gastric hydrochloric acid, and presence of various inhibitors and enhancers of iron absorption (4,5).

No recent comprehensive national study for the assessment of iron status has been conducted in Jordan. Studies on samples of a limited number of Jordanian subjects were based on blood hematocrit or hemoglobin only. Unfortunately, neither hematorcit nor hemoglobin is a specific indicator for evaluating iron status and thus cannot be used as proxy indicators of iron deficiency. Serum ferritin is the indicator of choice for measuring iron deficiency. The present study is based on serum iron and serum ferritin.

Iron status has been reported in different populations (6-12), but little information is available about this biological parameter among healthy Jordanians. Knowledge of the prevalence of iron status among females and males living in Jordan is of great importance, as iron status has an implication for general health. In-

cluding those two populations with varied age groups in the study can add more clarification on risk factors that could be associated with iron deficiency. Although iron status has assumed pandemic proportions all over the world, there are no reliable data on the iron status of the local healthy population in Jordan. Therefore, the presents work was undertaken to evaluate iron status among males and female, then, to trace variables may affect circulating iron status.

Materials and Methods

Subject selection

Serum blood samples were taken from two hundred and frothy healthy subjects aged between 19-65 years-with an average of 33.2±15.27 years- during routine laboratory check up at Zarqa governorate hospital. Subjects already had IDA or ID, had hematological and oncological disorders, took vitamin or mineral supplementation, and had family history of chronic disease leading to iron malabsorption, such as celiac disease were excluded. Furthermore, subjects under continuous or recent medication, which are known to influence iron status metabolism, women taking hormonal contraception or any other form of hormone substitution, were also excluded. Subjects were instructed to fast at least 12 hours before collecting the blood samples; qualified laboratory technicians collected the blood samples from the subjects.

Ethical consideration

All the study participants gave voluntary informed written consent before participation. Those who refused to take part in the study were excluded. The research was performed according to the Jordanian declaration.

Determination of serum ferritin and iron concentration

Blood samples were centrifuged within 1 hr at the survey site at 3000 x g for 5 minutes and then an aliquot of serum was frozen at – 20 *˚C* and then used for the determination of Serum ferritin concentrations using standard commercial Abbott kits, the AxSYM Ferritin Assay for the fully automated Abbott AxSYM system (Abbott Diagnostic Division, Longford, Ireland). AxSYM Ferritin assay is a microparticle enzyme intrinsic factor assay (MEIA) for quantitative determination of ferritin in human serum or plasma. The AxSYM Ferritin assay is designed to have precision of ≤ 9% total CV for concentrations within the ferritin low, medium and high control ranges, and designed also to have a sensitivity of 1ng/mL at 95% quantile. The assay was conducted according to the manufacture's instruction using AxSYM analyzer. The ferritin cut off concentrations of <13µg/I were considered to indicate absent iron stores according to WHO recommendations. Serum ferritin concentrations of 16-32 µg/I and 33-300 µg/I were considered as indicator for small and replete iron stores respectively, whereas concentrations of >300 µg/I and >700 µg/I were considered as indicator for moderate and heavy iron load respectively. Serum iron was determined calorimetrically using the FerroZine method on the COBAS INTEGRA 400 (Iron, 0-058) analyzer (Roche Diagnostic GmbH, Indianapolis, IN, USA). The procedures were carried out according to the manufacturer's guidelines. Individuals with concentrations below the cut-off value (<60 ng/mL) were considered to have ID. All these procedures pertaining to serum ferritin and serum iron were carried out at the Clinical Diagnostic Laboratory, Alkhaldi Medical Centre, Amman.

Statistical analysis

Chi-square test was used for comparing the prevalence of iron and ferritin status according to gender and age group in all subjects. Mean values of serum iron and serum ferritin were obtained by age and gender. Descriptive analysis was done for laboratory tests, and proportions were determined. Mean of the study variables between groups were compared using analysis Post hoc Tukey's test to observe any changes them. The differences were considered significant at P- values of less than 0.05. All Statistical analysis was conducted using the SPSS software (version 16) (SPSS Inc., Chicago, USA).

Results

The true prevalence of ferritin and iron status

Average age of the group studied was 33.2±15.27 years ranged from 19 to 65 years. Table 1 summarizes the personal habits and general health of the study population. Prevalence of ferritin and iron status in the two genders is shown in Table 2. There were 86 (35.8%) males and 154 (64.2%) females. The prevalence of serum ferritin concentrations (<16μg/l) was 3.4% (n=8) in males and 24.1% (n=58) in females. Serum ferritin concentration of 16-32 μg/l were present in 19.6 % (n=47) of the study population. 50.8 % (n=122) of participants had normal ferritin concentrations (33-300ug/l), but high serum ferritin concentrations (>300μg/l) were detected in 2.1% (n=5), while serum ferritin concentrations (>700μg/l) was not observed in the present study. However, the prevalence of serum iron concentrations (<60μg/l) was 5.4% (n=13) in males and 18.8% (n=45) in females (P=0.01).

Significant differences were found in the prevalence of ferritin concentrations and gender stratified by age as shown in Figure 1A and Figure 1B; approximately 14.6% (n=35) of females over 25 year old age group had significantly higher prevalence of ferritin deficiency (<16μg/l) compared with 0.84% (n=2) of males, however, 9.62% (n=23) of females and 2.4% (n=6) of males below 25 year old age group were ferritin deficient. The prevalence of serum ferritin concentrations (>300 μg/l) appeared to be similar for both males and females below 25 year old age groups, while serum ferritin concentrations of (16-32 μg/l) was significantly higher in females than in males in both age

Table 1. Description of the personal habits and general health among study population.

Personal habits and general health	Variable	n	(%)
Nutritional Status	Vegetarian	3.00	(1.20)
	Non-vegetarian	237	(98.8)
Current Medication from GP	Yes	6.00	(2.70)
	No	234	(97.3)
Current Nutritional Supplement	Yes	9.00	(3.90)
	No	231	(96.1)
General Health during the last 12 Months	Better than average	55.0	(22.9)
	Worse than average	44.0	(18.6)
	About the same	141	(58.5)

Table 2. The Prevalence of ferritin and iron deficiency among study population.

Variable		Male	Female n (%)	Total n (%)	p-value n (%)
Serum Ferritin (μg/l)	< 13	5(2.1)	50(20.8)	55 (22.9)	<0.05
	< 16	3(1.3)	8(3.3)	11(4.6)	
	16 – 32	12(5.1)	35(14.6)	47(19.6)	
	33 – 300	63(26.3)	59(24.6)	122(50.8)	
	301 - 700	3(1.3)	2(0.83)	5(2.1)	
	Total	86(35.8)	154(64.2)	240	
Serum Iron (ng/ml)	< 60	13(5.4)	45(18.8)	58(24.2)	0.01
	> 60	73(30.4)	109(45.4)	182(75.8)	
	Total	86(35.8)	154(64.2)	240	

Notes: Groups analyzed by Pearson chi-square test (2-sided); statistical significance was taken as p<0.05. Iron status was significant higher among females compared with males (p<0.05; p=0.01).

groups. About 65.4% (157/240) of the study population was older than 25 years; 15.4% (37/240) of this group were ferritin deficient as shown in figure 1-b.

Risk of serum iron deficiency tended to be significantly higher in females than in males at the different age groups (P=0.09 and P=0.06). Prevalence of iron status according to the two genders and age groups are shown in Figure 2A. About 15.8% (38/240) of the study population was below than 25 years; 10.4% (25/240) of this group were serum iron deficient (<60 ng/mL) as shown in Figure 2B.

The mean serum concentrations of ferritin and iron status

The mean serum concentrations of iron and ferritin were compared for both males and females in both age groups. The results shown in Table 3A and Table 3B show that the mean serum concentrations of ferritin in the range from (<13 to >300 µg/l) and iron in the range from (<60 to>60 ng/mL) was not affected by sex group, although mean values of ferritin and iron appeared to be lower for females than males, the same affect was true for the interaction between sex and age group with corresponding ferritin concentrations respectively (P=0.69) as shown in Table 2B. However, The mean serum concentrations of iron was affected by age (P=0.03), females below 25 year old age group had significantly lower iron concentration compared with males below 25 year old, the same affect was true for males and females over 25 year old age group.

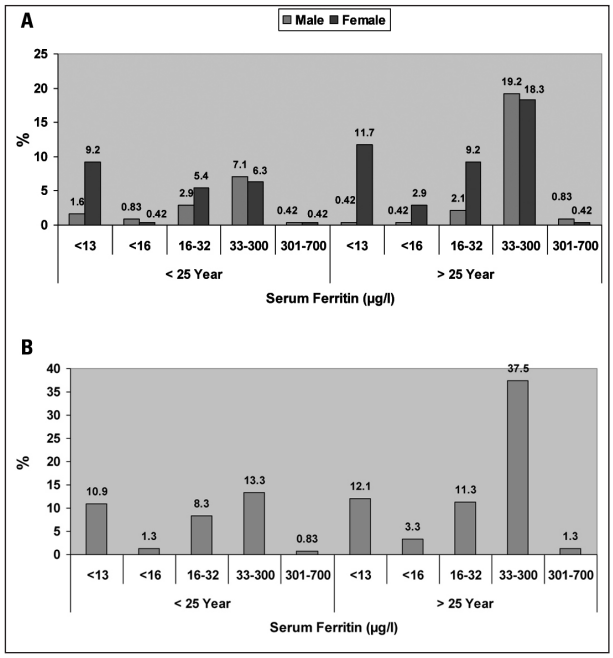

Figure 1. A) Ferritin status among males and females stratified by sex and age group. B) Ferritin status among the study population stratified by different age group.
Notes: Groups analyzed by Pearson chi-square test (2-sided); statistical significance was taken as p<0.05. Ferritin deficiency was significant higher among younger (p=0.04 ;< 25 year) and older (p=0.00; >25 year) age females (n=86) compared with all males (n=154).

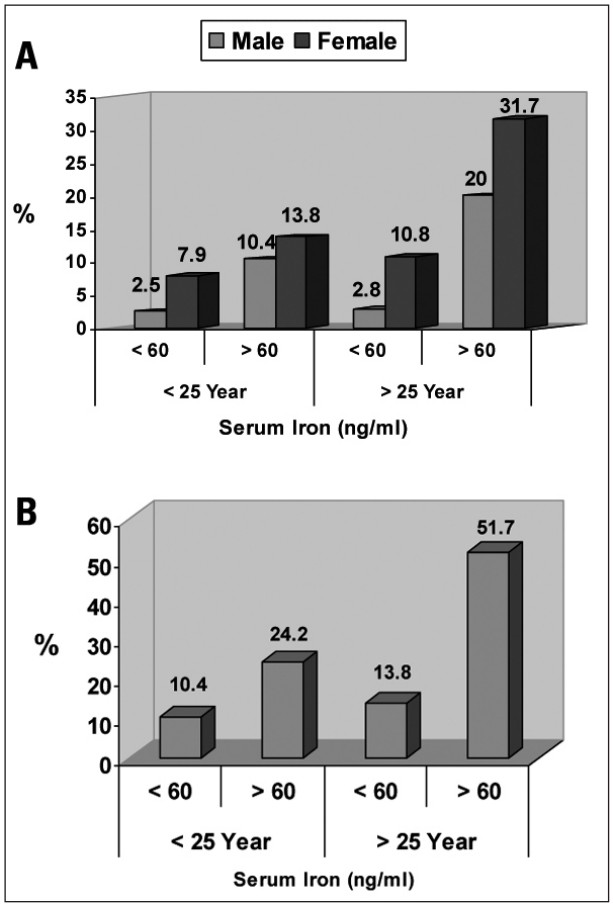

Figure 2. A) Iron status among males and females stratified by sex and age group. B) Iron status among the study population stratified by different age groups.
Notes: Groups analyzed by Pearson chi-square test (2-sided); statistical significance was taken as p<0.05. Iron deficiency was not significant higher among younger and older age females (p=0.09; n=86) compared with all males (n=154).

Table 3a. Mean serum concentrations of ferritin and iron among the study population stratified by gender.

Variable		Male mean ± SD	Female mean ± SD	*p*-value
Serum Ferritin (µg/l)	< 13	8.5±3.76	8.3±4.311	0.46
	< 16	15.2±0.45	14.5±0.91	
	16 – 32	25.5±5.26	24.3±4.62	
	33 – 300	111.1±77.51	89.3±63.25	
	301 - 700	410.0±171.90	382.95.36	
	Total	117.7±110.13	101.6±100.55	
Serum Iron (ng/ml)	< 60	51.1±8.28	41.3±13.66	0.78
	> 60	129.5±115.61	126.4±110.1	
	Total	100.2±100.47	48.2±66.39	

Table 3b. Mean serum concentrations of ferritin and iron among the study population stratified by age and gender.

Variable		Male mean ± SD	Female mean ± SD	*p*-value
Serum Ferritin (µg/l)	< 25 years	98.4±43.06	98.5±109.54	0.69
	> 25 years	128.5±133.15	103.1±100.55	
	Total	117.7±110.13	101.6±100.55	
Serum Iron (ng/ml)	< 25 years	69.3±77.94	40.6±60.73	0.03
	> 25 years	117.7±107.96	52.1±69.07	
	Total	100.2±100.4	48.2±66.39	

Notes: Groups analyzed by post Tukey's test; statistical significance was taken as p value<0.05; serum iron status was significantly higher among males compared females across all age groups (p=0.03) as shown in Table 2B. However, the mean concentration of serum ferritin was not affected by gender, age and their interaction (p<0.05) as shown in Table 2A.

Discussion

The present study demonstrated that Ferritin deficiency was significant higher among younger and older age females compared with all males. Furthermore, serum iron deficiency appeared to be similar for both males and females according the different age groups, while risk of iron deficiency was significantly higher in females than in males for the study population. These findings suggest that women may be considered at high risk of IDA if it is not treated in the early stage of the disorder (i.e. ID).

The findings of lower mean ferritin and iron concentrations in females compared to males can be attributed to blood losses in females during menstruation, although that difference did not reach a statistical level of significance. Furthermore, the observed higher mean iron concentration in males over 25 year-old compared with females at same age group which suggesting that the mean iron concentration was affected by age. While the mean ferritin concentration was similar in the two age groups for both gender. An explanation for such a finding may point to that Jordanian males eat higher daily intake of meat than females, this can be attributed to meat which contains not only the well-absorbed heme iron, but also a peptide (sometimes called the meat factor) that enhance nonheme iron absorption. These results agree with previous reports (13,14).

The mean value of serum ferritin for the studied population (66.8±83.89 µg/l, n=240) is notably higher than what other surveys reported (6-13), indicate that Jordanians have normal level of ferritin status. In addition, the present study observed that the prevalence of

low ferritin concentration was 13.8% of the study population. Probably low prevalence of low ferritin concentration in the studied population caused an increase in the mean of ferritin concentration. In other words, although the mean ferritin concentration is high, the severity of iron deficiency remain low. Furthermore, ferritin concentrations compared to surveys carried out in many countries in this region, indicate that the prevalence of low ferritin concentrations were lower than other countries (6-12). These findings suggest that Jordanians eat adequate intake of animal products such as meat, poultry and fish to increase the daily intake of iron. However, it is possible that there may be other important dietary factors that may effect non-heme iron absorption like tea and coffee consumption (15-17), phytates in legumes, grain and rice (18), the vegetable protein in soybeans, legumes, and nuts (5), the calcium in milk (5), The MFP factor (19), some acids (e.g. ascorbic acids, citric acid and lactic acid) and sugars (e.g. fructose) (5), an adequate daily intake of iron, vitamin B12 and folic acid (11), inflammatory diseases (6), the use of vitamin –mineral supplements containing iron (13), that were not taken in consideration in this study that could influence the iron status of those subjects this warrants further study.

Finally, in order to correlate our findings to the dietary intake of iron and the bioavailability of dietary iron it will be important to calculate iron intake in the diet consumed in Jordan using food composition tables and chemical analysis. It would also be interesting to assess the iron content of hospital diet, mixed and vegetarian diets as well as plant-derived and animal- derived foods since there is little information available concerning these items. Since it is emerging that ID influence behavior (5), it would be useful to study the effects of ID on children's and adult's behavior. Furthermore, it would be interesting to establish whether genetic polymorphisms in hemochromatosis genes (e.g. homozygosity of C282Y) in combination with dietary factors such as the daily intake of animal product associated with iron absorption in the small intestine (20,21).

The present study has some important limitations. First, this study based on serum iron and ferritin results, which alone, without biochemical markers such as hemoglobin, hematocrit, transferrin or iron binding capacity, that can determine the severity of deficiency. Second, the smaller sample size of the studied population. Third, the current study also did not calculate the dietary intake characterized by age of introducing foods which considered to be more appropriate for identifying the dietary risk factor that associated with ID occurrence.

Conclusion

In conclusion, iron status was satisfactory in both males and females, since the prevalence of normal ferritin were observed in 24.6 % of females and 26.3% in males of the entire series, which was higher than what other surveys reported, these result suggest that Jordanian males eat higher intake of animal product such as meat, poultry and fish than females, which is adequate to maintain a favorable iron status with a low prevalence of ID.

Acknowledgements

The author kindly thanks Dr. Islam Al-shami, the Hashemite University, Jordan, for her cooperation through this study.

References

1. DeMaeyer E, Adiels-Teyman M. The prevalence of anemia in the world. World Health Stat Q, 1985; 38:303–16.
2. World Health Organization. The prevalence of anaemia in women: a tabulation of available information. 2nd ed. Geneva: World Health Organization; 1992.
3. Beard L J. Iron requirements in adolescent females. J Nutr 2000; 130: 440S–2.
4. Denic S, Agarwal MM. Review article, nutritional iron deficiency; an evolutionary perspective. Nutrition, 2007; 23: 603-614.
5. Whitney E, Rolfes S R,Understanding nutrition, 13th ed ; Wadsworth, Cengage learning, USA.
6. Akramipour R, Rezaei M, Rahimi Z. Prevalence of iron deficiency anemia among adolescent schoolgirls from Kermanshah, Western Iran. Hematology, 2008, 13: 352-5.
7. Shams S, Asheri H, Kianmehr A, Ziaee V, Koochakzadeh L, Monajemzadeh M, Nouri M, Irani H, Gholami N. The prevalence of iron deficiency anaemia in female medical students in Tehran. Singapore Med J, 2010, 51: 116-9.
8. Abalkhail B, Shawky S. Prevalence of daily breakfast intake,

iron deficiency anaemia and awareness of being anaemic among Saudi school students. Int J Food Sci Nutr, 2002, 53: 519-28.
9. Al-Quaiz JM. Iron deficiency anemia. A study of risk factors. Saudi Med J, 2001, 22: 490-6.
10. Al-Othaimeen A, Osman AK, al Orf S. Prevalence of nutritional anaemia among primary school girls in Riyadh City, Saudi Arabia. Int J Food Sci Nutr, 1990, 50: 237-43.
11. P Mirmiran, M Golzarand, L Serra-Majem, Review Article Iron, Iodine and Vitamin A in the Middle East; A Systematic Review of Deficiency and Food Fortification, Iranian J Publ Health, Vol. 41, No.8, Aug 2012, pp. 8-19.
12. Rougereau A, Gore' J. N'diaye M, Person O, ferritin and iron status in Senegalese women, The American Journal for clinical nutrition, 36,1982, 314-318.
13. Milman N, Pedersen AN, OvesenL, Schroll M. Iron status in 358 apparently healthy 80-year-old Danish men and women: relation to food composition and dietary and supplemental iron intake, Ann Hematol, 2004, 83:423–429.
14. Skikne R, Baynes RD (1994) Iron absorption. In: Brock JH, Halliday JW, Pippard MJ, Powell LW (eds) Iron metabolism in health and disease. Saunders, London, pp 151–187.
15. Skikne R, Baynes RD (1994) Iron absorption. In: Brock JH,Halliday JW, Pippard MJ, Powell LW (eds) Iron metabolism in health and disease. Saunders, London, pp 151–187.
16. Milman N, Ovesen L, Byg K-E, Graudal N (1999) Iron status in Danes updated 1994. I: prevalence of iron deficiency and iron overload in 1332 men aged 40–70 years. Influence of blood donation, alcohol intake, and iron supplementation. Ann Hematol 78:393–400.
17. Milman N, Ovesen L, Byg K-E, Graudal N. Iron status in Danes 1994. II: prevalence of iron deficiency and iron overload in 1319 Danish women aged 40–70 years. Influence of blood donation, alcohol intake, and iron supplementation. Ann Hematol, 2000, 79:612–621.
18. Sadighi J, Mohammad K, Sheikholeslam R, Amirkhani MA, Torabi P, Salehi F, Abdolahi Z. Anaemia control: lessons from the flour fortification programme. Public Health, 2009, 123: 794-9.
19. Skikne R, Baynes RD (1994) Iron absorption. In: Brock JH, Halliday JW, Pippard MJ, Powell LW (eds) Iron metabolism in health and disease. Saunders, London, pp 151–187.
20. Pietrangelo A. Hereditary hemochromatosis-a new look at an old disease. N Engl J Med, 2004; 350: 2383–97.
21. Datz C, Haas T, Rinner H, Sandhofer F, Patsch W, Paulweber P. Heterozygosity for the C282Y mutation in the hemochromatosis gene is associated with increased serum iron, transferrin saturation,and hemoglobin in young women: a protective role against iron deficiency? Clin Chem, 1998; 44: 2429 –32.

Correspondence:
Ala Ali Qatatsheh
Department of Nursing, Princess Aisha Bint Al-Hussein Faculty of Nursing, Al-Hussein bin Talal University, Ma'an, Jordan and Department of Clinical Nutrition,
Faculty of Applied Health Sciences,
Qassium University, Arrass, Saudi Arabia
E-mail: seif1972@yahoo.com