

DETERMINATION OF SERUM IRON, TOTAL IRON-BINDING CAPACITY AND SERUM FERRITIN IN HEALTHY SAUDI ADULTS

Ahlan M. Al-Buhairan, M.Sc; Olayide A. Oluboyede, MD

It has long been a well-established practice to determine the normal values of various hematological parameters in different parts of the world. Published values of serum iron (SI), total iron-binding capacity (TIBC), percentage transferrin saturation and serum ferritin in apparently healthy population groups vary significantly from one country to another.¹⁻⁵ Besides the studies of El-Hazmi et al.⁶ and Bacchus et al.,⁷ there are no other reports on these values from the Kingdom of Saudi Arabia. This study was undertaken because of the limited information available on these parameters, and the fact that reference values from Western countries, which have different environmental conditions and nutritional habits from Saudi Arabia and other tropical countries, are currently used to determine the values of these hematological parameters.

Materials and Method

Blood samples were collected from 300 healthy Saudi adults, comprising 150 males and 150 females living in the Riyadh region, in the Central Province of Saudi Arabia. They were selected from university students, academic and non-academic staff of King Saud University, employees from different professions at King Faisal Specialist Hospital and Research Centre (KFSH&RC), Riyadh, and volunteers from the general public. Approximately 34% of females were married, 63% of the males were smokers, while all the females were nonsmokers. The selection of the volunteers who were apparently healthy individuals was based on the following criteria: age range between 20-40 years; no history of drug usage (including vitamins/iron, antibiotics); no recent history of blood loss; and must not have received any blood transfusion in the previous 12 months. Additional criteria for females included not being pregnant, and not lactating or menstruating at the time of blood collection. The blood was drawn in the morning between 8 and 11 am.

Before starting collection of blood, the volunteer recorded information on age, sex, medical history, drug usage, smoking history and stage of menstrual cycle. While in an upright position, the tourniquet was applied for a few seconds, and the venous blood was drawn by means of venipuncture (vacutainer system with multiple sample needles was used). The usual precaution of selecting an easily accessible vein in the antecubital fossa and applying the minimum of venous stasis was observed.

A total of 10 cc of blood was collected from each volunteer, and 7 cc of it was transferred into a clean, screw-capped glass tube without anticoagulant and allowed to stand at room temperature until separation of the serum was started by centrifugation at 4°C, at a speed of 3000 rev/min. The separated sera were kept at 20°C until analyses were done. Analyses conducted on the sera were the determination of serum iron, serum ferritin and calculation of total iron-binding capacity of transferrin from the value of transferrin. The analysis was done at the Department of Pathology and Laboratory Medicine, Hematology and Biochemistry Sections, at KFSH&RC. Before the analysis, the separated frozen sera were placed at room temperature to allow thawing, and were then mixed.

Measurement of Serum Iron

SI concentration was measured by the Forrozine method, and the assay was carried out using BM/Hitachi 717. Before running the assay, the analyzer was calibrated by STD2, which is a serum-based calibrator. To assess the accuracy of the analyzer, Precinorm U and Precipath U controls were run with the assay in addition to precinorm UPX control, which was used to assess precision.

Calculation of TIBC and Transferrin Saturation Fraction

Transferrin was measured first in order to calculate the TIBC of transferrin and later transferrin saturation fraction. The assay of transferrin was carried out using the Cobas Mira Plus Analyzer. Human transferrin forms a precipitate with a specific antiserum, which is determined turbidimetrically at 340 nm. TIBC and TS were then calculated according to the following formulae:

$$\text{TIBC} = \text{Transferrin} \times 24$$

$$\text{TRSF} = \frac{\text{Serum Iron Concentration} \times 100}{\text{TIBC}}$$

From the Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia.

Address reprint requests and correspondence to Prof. Oluboyede: Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Saud University, P.O. Box 10219, Riyadh 11433, Saudi Arabia.

Accepted for publication 11 October 2000. Received 13 February 2000.

TABLE 1. Values of serum iron, TIBC, transferrin saturation percentage and serum ferritin in healthy Saudi males and females.

Parameter	Males (n=149)					Females (n=134)					P-value
	Mean	SD	Percentile*			Mean	SD	Percentile*			
			2.5	50	97.5			2.5	50	97.5	
Serum iron ($\mu\text{mol/L}$)	17.20	5.70	8.00	17.00	31.20	13.30	5.30	5.00	12.00	25.60	<0.05
TIBC ($\mu\text{mol/L}$)	63.40	8.60	48.00	63.00	81.00	74.30	11.70	54.80	73.00	101.30	<0.05
Transferrin saturation fraction (%)	0.27	0.10	0.11	0.26	0.54	0.18	0.08	0.07	0.17	0.42	<0.05
Serum ferritin ($\mu\text{g/L}$)	92.40	2.10	18.00	94.00	350.00	21.20	2.20	7.00	21.90	110.00	<0.05

*2.5th and 97.5th percentiles represent the lower and upper limit of the range, respectively.

Prior to assay, the instrument was calibrated with the use of Serum Proteins T standard. The instrument was controlled via BioRad Liquichek Immunology Control, levels 1 and 2.

Measurement of Serum Ferritin

Serum ferritin level was measured by the Ciba-Corning Automated Chemiluminescence System (ACS 180), employing a two-site chemiluminometric (sandwich) immunoassay, which uses constant amounts of two anti-ferritin antibodies. The ACS 180 was calibrated using master curve and a two-point calibration. The assay was controlled by BioRad Lymphochek; three levels were used (I, II, III) at the beginning before assaying any samples, and all three levels were assayed whenever a calibration was performed. Also, the performance of the ACS 180 was checked by assessing the accuracy and precision of the ACS ferritin assay. Four sera samples were assayed three times.

The statistical analysis of the data was carried out by JMB Power Macintosh Microsoft program (1995). Arithmetic mean, media, standard deviation, standard error of mean, 2.5th and 97.5th percentiles, and skewness were obtained. Frequency distribution histograms were plotted for most of the parameters by personal computer, using Microsoft Excel version 7.0. The data analysis was conducted separately for each sex and the Student's *t*-test, assuming equal variances and the non-parametric (Wilcoxon's) *z*-test were used to determine the statistical significance of the difference in the mean values for Saudi males and females. A *P*-value of <0.05 was considered significant.

The reference range (95% range) value was calculated for each parameter by either the standard parametric method, i.e., mean \pm 2 SD for those parameters which exhibit the Gaussian distribution or an approximation of the Gaussian distribution. On the other hand, the non-parametric approach was used to calculate the reference range when the data were skewed or not normally distributed. The 2.5th and 97.5th percentiles were used to present the lower and the upper limits of the range.

Results

Values of SI, TIBC and percentage transferrin saturation for Saudi males and females are shown in Table 1. The mean SI value in Saudi males was significantly higher than in the females ($P<0.05$), and the value was more positively skewed in the males than in females. Values of the TIBC were higher in females than in males. The difference between the two sexes was statistically significant ($P<0.05$). The distribution of the values was more positively skewed in the female group.

Percentage transferrin saturation values were higher in the males than in females. The difference between the two sexes was statistically significance ($P<0.05$). The values of serum ferritin levels in Saudi males and females are presented in Table 1. The mean serum ferritin value in the males (92-40 $\mu\text{g/L}$) was higher than that in the females (21.20 $\mu\text{g/L}$). The difference between the two groups was statistically significant ($P<0.05$).

Discussion

The values of SI concentration obtained for both sexes in this study are lower than values obtained in a previous study by El-Hazmi et al. of male and female students of King Saud University.⁶ The difference is probably due to different samples studied. The students in the previous study were aged 20-29 years, whereas in our study, the subjects were aged 20-40 years. However, the results of our study are not different from those obtained by Bacchus et al.⁷ The study was done on 1376 Saudi males and females, with a wider age range varying from 18-60 years. The range values of TIBC in males in our study are also similar to those in the previous study by El-Hazmi et al., however, the range values in females in our study were much wider.⁶

In a study by Jacobs et al.,² values obtained for SI and transferrin saturation among a healthy Welsh population of both sexes were higher than those in our study. The values obtained in this study are also lower than those found in an American study,⁵ which had a mean transferrin saturation value of 31.2% in the female population compared to 18% in ours. The TIBC value in males, however, was similar to the value in males in our study. In an adult female Canadian population,³ the mean value of transferrin saturation was higher than the value in females in our study, however, the transferrin saturation values in males

in both studies were similar. In this study, Valberg et al.³ reported higher serum iron and TIBC mean values in the female population than in females in our study.

From Nigeria, Oluboyede et al.⁴ reported higher serum iron, TIBC and transferrin saturation values than those in our study. The mean TIBC values of 77.7 $\mu\text{mol/L}$ and 89.3 $\mu\text{mol/L}$ for Nigerian males and females, respectively, were higher than the mean values of 63.4 $\mu\text{mol/L}$ and 74.3 $\mu\text{mol/L}$ for Saudi males and females, respectively.⁸ In a study by Kuvibidila,⁹ Zairean non-lactating women showed higher values for SI, TIBC and TS than values obtained in Saudi women. Perry et al.¹⁰ have reported a transferrin saturation value of 24.3% in black American males, which was lower than the value of 27% in males in our study. In the females, however, transferrin saturation values of 17.84% and 18.42% in black and white Americans, respectively, were similar to the mean values of 18% obtained in females in our study.

Dacie and Lewis¹¹ reported a single range value of 13-32 $\mu\text{mol/L}$ for serum iron (for both male and female), transferrin saturation range value of 28-45 (both sexes), and TIBC range value of 45-70 $\mu\text{mol/L}$ (both sexes). Wintrobe¹² reported higher serum iron values of 12.7-35.9 $\mu\text{mol/L}$ in males and 11.0-30 $\mu\text{mol/L}$ in females than in our study, however, our TIBC range values in this study were higher in both sexes. The SI values of 11.64-30.43 $\mu\text{mol/L}$ in females reported by Tietz and Rinker¹³ were higher than values obtained in Saudis. Values for transferrin saturation in males (20-55%) and in females (15-50%) were also higher.

There are many possible explanations for the differences in the findings in this study and those of other studies. Bias in the selection of subjects could be an explanation. Cook et al.,⁵ for example, used a selected group in which anemia and iron deficiency were excluded. Jacobs et al.,² who reported lower values, used an unselected group. The time of sample collecting also affects the concentration value of serum iron. There is marked diurnal variation in the values of serum iron concentration, the values being highest in the morning, low in mid-afternoon, and lowest near midnight.¹⁴⁻¹⁶ The percentage of transferrin saturation is also variable since it is affected by the serum iron concentration. Lastly, differences might reflect true differences in the iron status of various groups. Saudi females had lower SI and transferrin saturation than their male counterparts. The reduced SI in Saudi females is probably due to low levels of dietary iron intake. This is made worse by monthly blood losses. As well, parasitic infections, e.g., hook worm infestations were not excluded.

Serum ferritin determination provides a direct measurement of total body iron stores,^{2,17} and thus helps in the differentiation between iron deficiency anemia and anemia due to chronic infection or inflammation. In this study, serum ferritin mean value of 92.4 $\mu\text{g/L}$ in males is significantly higher than the mean value of 21.2 $\mu\text{g/L}$ in

females ($P < 0.05$). The markedly higher value in males is consistent with the known observation of sex differences in the storage of iron.^{2,14,17,19}

The mean male value of 92.4 $\mu\text{g/L}$ in Saudis in our study is similar to the value of 94.0 $\mu\text{g/L}$ found among American males¹⁴ and also the 93.0 $\mu\text{g/L}$ reported among Canadians.³ The value in Saudi males is, however, higher than the value of 69 $\mu\text{g/L}$ found in Welsh males² and the 72.4 $\mu\text{g/L}$ found in Nigerian males.⁴ The high value of serum ferritin in Saudi males may be a reflection of adequate iron stores, probably due to high levels of meat consumption. The significant influence of meat intake on serum ferritin concentration among adults has been reported.²⁰ In Saudi females, the mean value of low serum ferritin of 21.2 $\mu\text{g/L}$ and range of 7-104.5 $\mu\text{g/L}$ are close to the mean and range values of 23 $\mu\text{g/L}$ and 5-104 $\mu\text{g/L}$ reported in 115 healthy Danish females.²¹

Low serum ferritin concentration indicates low body iron stores. In general, the iron status of Saudi females seems to be lower than in females in the Western countries. The reason may be due to low iron intake by Saudi women. The iron intake in a mixed population of male and female adults in the Riyadh region was reported to be 47.1 mg/day.²² How much of this iron is available to the body is unknown. More studies are needed to define the relative contributions of these factors as they affect iron status of Saudi females.

Acknowledgements

The authors would like to express their gratitude to Dr. Ashraf Ali, Distinguished Senior Consultant, Pathology Department at KFSH&RC, for his cooperation in the collection and analysis of blood samples. We would also like to thank Dr. Gamal El Din Mohammed of the Biochemical Statistics and Scientific Computing Department at KFSH&RC for the statistical assistance, and finally, all the volunteers without whom this study could not have been done.

References

1. Sinniah R, Neil DW. Serum iron, total iron-binding capacity and percentage transferrin saturation in normal subjects. *J Clin Path* 1968;21:603-10.
2. Jacobs A, Miller F, Worwood M, Beamish MR, Wardrop CA. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *BMJ* 1972;4:206-8.
3. Valberg, LS, Scobie J, Ludwig J, Pelletier O. Serum ferritin and the iron status of Canadians. *Can Med Assoc J* 1976;114:417-21.
4. Oluboyede OA, Usanga EA, Lukanmbi FA, Ajayi OA. Evaluation of serum ferritin levels and other haematological parameters in a Nigerian population. *J Nat Med Assoc* 1983;75:885-9.
5. Cook JD, Lipschitz DA, Miles LEM, Finch CA. Serum ferritin as a measure of iron stores in normal subjects. *Am J Clin Nutr* 1974;27:681-7.
6. El-Hazmi MAF, Al-Faleh FZ, Al-Mofleh IA, Warsy AS, Al-Askah AK. Establishment of normal reference range for haematological parameters for healthy Saudi Arabs. *Trop Geog Med* 1982;34:333-9.

7. Bacchus R, Garmer E, Madkour MM, Haque S, Hardle A. The haematology reference range for Saudi Arabians. *Saudi Med J* 1986;7:46-52.
8. Al-Buhairan AM, Khalil SH, Oluboyede OA. Reference range values of haematological parameters in Saudi healthy adults. *Saudi Med J* 1999;20:757-62.
9. Kuvibidila S, Yu L, Warriar RP, Ode D, Mbele V. Usefulness of serum ferritin levels in the assessment of iron status in non-pregnant Zairean women of childbearing age. *J Trop Med Hyg* 1994;97:171-9.
10. Perry GS, Byers T, Yip R, Margen S. Iron nutrition does not account for hemoglobin differences between blacks and whites. *J Nutr* 1992; 122:1417-24.
11. Dacie JV, Lewis SM. *Practical Haematology*. 7th edition. London: Churchill Livingstone, 1991.
12. Wintrobe MM. In: Lea GR, Bithell TC, Foerster J, Athens JW, Lukens JN, editors. *Wintrobe's Clinical Haematology*. Volume 2. 9th edition. Philadelphia: Lea and Febiger, 1993.
13. Tietz NW, Rinker AD, Morrison SR. When is serum iron really a serum iron? The status of serum iron measurements. *Clin Chem* 1994;40:546-51.
14. Cook JD, Finch CA, Smith NJ. Evaluation of the iron status of a population. *Blood* 1976;48:449-55.
15. Statland BE, Winkel P. Relationship of day-to-day variation of serum iron concentration to iron-binding capacity in healthy young women. *Am J Clin Pathol* 1977;67:84-90.
16. Pilon VA, Howanitz PJ, Howanitz JH, Domres N. Day-to-day variation in serum ferritin concentration in healthy subjects. *Clin Chem* 1981;27:78-82.
17. Walters GO, Miller FM, Worwood M. Serum ferritin concentration and iron stores in normal subjects. *J Clin Pathol* 1973;26:770-2.
18. Vicente C, Porto G, Sousa M. Method for establishing serum ferritin references values depending on sex and age. *J Lab Clin Med* 1990;116: 779-84.
19. Leggett BA, Brown MM, Bryant SJ, Duplock L, Powell LW. Factors affecting the concentrations of ferritin in serum in a healthy Australian population. *Clin Chem* 1990;36:1350-5.
20. Soustre Y, Galan P, Hereberg S. Dietary determinants of the iron status in menstruating women. *Int J Vit Nutr Res Suppl* 1986;56:281-6.
21. Milman N, Andersen HC, Padersen NS. Serum ferritin and iron status in healthy elderly individuals. *Scand J Clin Lab Invest* 1986;46:19-26.
22. Al-Kanhal MA, Al-Othaimeen AI. Evaluation of the nutritional status of the people of Saudi Arabia. KACST (Final Report), 1994.