SPANDIDOS PUBLICATIONS

EXPERIMENTAL AND THERAPEUTIC MEDICINE 25: 218, 2023

A reference interval study of ferritin and transferrin levels in donors from two blood centers

XIANFEI ZENG[1,2*], XI MENG[2,3*], YAFEN WANG[4*], SHIJIE LI[5], QIAO LIAO[5], ZHENGANG SHAN[5], JIETING HUANG[5], JIYUAN SHI[4], LIWEI WANG[1], HONGYAN CHEN[2], XINGBIN HU[4] and XIA RONG[5]

[1]School of Medicine, Northwest University, Xi'an, Shaanxi 710069; [2]Core Laboratory, Xi'an Area Medical Laboratory Center, Xi'an, Shaanxi 712000; [3]School of Basic Medicine, Xi'an Jiao Tong University, Xi'an, Shaanxi 710049; [4]Department of Transfusion Medicine, Xijing Hospital, Xi'an, Shaanxi 710032; [5]Institute of Clinical Blood Transfusion, Guangzhou Blood Center, Guangzhou, Guangdong 510095, P.R. China

Received November 3, 2022; Accepted March 2, 2023

DOI: 10.3892/etm.2023.11916

Abstract. Blood donors not only save the lives of patients but also play an important role in the development of medical and health services. Therefore, it is particularly important to pay attention to the blood health of blood donors who are at a high risk of iron deficiency. Detection of serum ferritin and transferrin is an important basis for the diagnosis of iron deficiency anemia. However, to the best of our knowledge, the levels of serum ferritin and transferrin, and the influencing factors, such as age and type of donation, in blood donors have not been clarified. In the present study, the serum ferritin and transferrin levels of donors from two blood centers were investigated. Demographic data were collected from the donors, and their serum ferritin and transferrin levels were tested. A total of 1,817 donors were enrolled and were eligible for evaluation. Reference intervals (RIs) for ferritin and transferrin were obtained from blood donors, and it was revealed that the ferritin and transferrin levels of blood donors were associated with age. Furthermore, serum transferrin levels were associated with the type of donation; the serum transferrin RI level was significantly higher in platelet-only donors compared with in whole blood donors. It was also demonstrated that ferritin levels were negatively associated with transferrin levels. The present study identified RIs for ferritin and transferrin levels in blood donors, and indicated that age and type of donation were important factors affecting ferritin and transferrin levels in blood donors. These findings may prove useful for blood donation recruitment and screening strategies in China, and could promote the health of blood donors.

Correspondence to: Professor Xingbin Hu, Department of Transfusion Medicine, Xijing Hospital, 15 Changle West Road, Xi'an, Shaanxi 710032, P.R. China
E-mail: hxbyqh@163.com

Professor Xia Rong, Institute of Clinical Blood Transfusion, Guangzhou Blood Center, 31 Luyuan Road, Guangzhou, Guangdong 510095, P.R. China
E-mail: joyjoy@126.com

*Contributed equally

Key words: reference intervals, ferritin, transferrin, blood donors

Introduction

Iron is an essential trace element for a variety of physiological functions in the human body, including oxygen transportation, metabolism, DNA synthesis and damage repair, neurotransmitter production, and transcriptional regulation (1,2). Iron deficiency is a widespread nutritional deficiency worldwide (3,4). Severe iron deficiency can lead to iron deficiency anemia, heart failure, and ischemic heart disease (5-7). However, iron deficiency in blood donors who are at high risk of iron deficiency will cause more serious iron homeostasis disorder, lower hemoglobin level, hypochromic anemia, and high delayed blood donation rate than non-donors, and even threaten the health of blood donors (8-10). Iron monitoring is therefore critical for human health, especially blood donors. Appropriate monitoring of ferritin and transferrin levels in blood donors has been shown to be beneficial for the regulation of national blood donation policies, measures to prevent iron deficiency and anemia in donors, adjustment of donation intervals, donor health, and blood supply (11).

Monitoring of iron in humans is usually reflected by the level of serum ferritin (12). Transferrin, the circulating iron-binding protein, is another key marker of iron status because its levels increase with iron deficiency (13). Other studies have shown that ferritin and transferrin levels may have a significant impact on blood donation and donor health (14). The current universally accepted cut-off value for the diagnosis of iron insufficiency is serum ferritin <15 ng/ml (excluding infection or inflammation) (15). However, the RIs and influencing factors for the levels of ferritin and transferrin in the diagnosis of iron deficiency in blood donors are not well established in China yet, although an iron deficiency in whole blood donors has been found to be much more common than expected, and regular platelet donation can also reduce ferritin levels and lead to iron deficiency in some developed countries (16-20).

There are only limited reports on the levels of ferritin and transferrin and the factors affecting iron metabolism in Chinese blood donors, and there is a lack of large sample studies from mainland China. No study has established ferritin and transferrin RIs following the protocols of the Clinical and Laboratory Standards Institute (CLSI) and International Federation of Clinical Chemistry and Laboratory Medicine, Committee on RIs and Decision Limits (IFCC/C-RIDL) (21-23).

Therefore, In the present study, the serum ferritin and transferrin level of whole blood and platelet donors from two blood centers was examined, and a 95% normal range as established for ferritin and transferrin of Chinese blood donors. The distribution pattern of ferritin and transferrin levels in Chinese blood donors of different ages and genders was explored. It was found that the ferritin and transferrin RIs of blood donors were associated with age. In addition, the type of donation influenced the level of transferrin, and the ferritin level was negatively correlated with the transferrin level. These findings can be used to establish more reasonable blood donation recruitment and screening strategies in China to protect the health of blood donors and promote voluntary non-remunerated blood donation.

Materials and methods

Donor and consent. Volunteer blood donors from Xi'an (North West China) and Guangzhou (South China) that met the Blood Donation Law of the People's Republic of China, were recruited in the present study. Requirements for blood donors: 18-55 years old; The weight of male donors should be at least 50 kg and that of female donors should be at least 45 kg. Donors should be free of colds and acute gastroenteritis for a week, without medication, and without alcohol for 24 h; The interval between two blood donations should be more than 6 months. Individuals who have a minor surgery within half a month, a general surgery within three months, and major surgery within six months, are not allowed to donation. Women should not donate blood during and 3 days before and after menstruation; Platelets should not be donated again before three months of whole blood donation; After 28 days of platelet donation, whole blood can be donated again. Donors were categorized into several groups according to age: the 18-22, 23-27, 28-32, 33-37, 38-42, 43-47, 48-52 and >52-year age groups. Donors who donated 3 times at intervals of <12 months were considered regular donors. According to the standards set by the World Health Organization, smokers are defined as those who have smoked continuously or cumulated for six months or more during their lifetime, and drinkers are defined as those who have used alcohol (beer, liquor, etc.) more than once in the past year (24,25). The study protocol was approved by the ethics committee of the Guangzhou Blood Center [approval no. Guangzhou Blood Center Lun (2020) No. 1]. Written informed consent was obtained from all recruited donors.

Inclusion and exclusion criteria. Donors who had lived in Xi'an or Guangzhou for >2 years were included in the present study. Donors who had been diagnosed with hemopathy or a metabolic disease were excluded.

Demographic information of recruited donors. In the present study, a total of 1,817 blood donor samples were used (Table I; Fig. 1), including 977 from Xi'an and 840 from Guangzhou. The study population comprised 1,410 males and 407 females, with a male-to-female ratio of 3.46:1. A total of 997 individuals donated whole blood and 820 donated apheresis platelets. Of those, 827 were first-time donors and 548 were regular blood donors who donated more than three times in <12-month intervals. The donation times increased with age. There was no significant difference in the average BMI among the age groups (P=0.248).

Blood sample preparation. All peripheral blood samples were obtained following the donation. Samples were collected in the dry tubes without anti-coagulant. The serum was then prepared by centrifugation. Serum was stored at -20˚C prior to testing.

Ferritin and transferrin test. According to current recommendations, the level of ferritin in the prepared serum was measured with a chemiluminescent microparticle immunoassay (Abbott i2000, Abbott Laboratories, USA). The transferrin concentration was determined by a turbidimetric inhibition immunoassay (ROCHE e701; Roche Diagnostics GmbH).

Statistical analysis. According to the data distribution, abnormal values were excluded by the Dixon method or Tukey's fence, as appropriate. An improved Box-Cox formula was utilized to convert the non-normally distributed data into normally distributed data. Multiple-regression analysis (MRA) and two-way nested ANOVA, based on Ichihara's method, was used to analyze age, gender and region difference (26). Two-way nested ANOVA was used to determine the necessity of partitioning reference values based on age, gender or region. Briefly, the standard deviation ratio (SDR) which represents the ratio of between-subgroup SD to between-individual SD was computed by two-way nested ANOVA. Based on the IFCC/C-RIDL protocol for establishing a RI, an SDR of >0.3 was viewed as a guide to consider stratifying reference by gender, age or region. Furthermore, a secondary exclusion procedure, latent abnormal values exclusion (LAVE), was used to eliminate potential iron metabolism abnormality (27). Briefly, the donors with levels of ferritin and transferrin simultaneously exceeding the established RIs were excluded from the calculation of the new reference ranges. A secondary round of exclusion was performed by excluding the enrolled donors with potential iron metabolism abnormalities to establish the new RIs based on data from the remaining participants. After six rounds of exclusion, the reference values were nearly stable. Blood type, body mass index (BMI), donation interval, donation times, drinking and smoking were also considered in the RI analysis. SPSS 19.0 (IBM Corp.) and GraphPad 8.0 (GraphPad Software Inc.) were used in the present study. P<0.05 was considered to indicate a statistically significant difference.

Results

Box-Cox transformation and SDR analysis. According to the Kolmogorov-Smirnov normality test, the ferritin (ng/ml) and transferrin (g/l) values were not normally distributed. Following Box-Cox transformation, the data of transferrin

Table I. Demographic information stratified by age.

Variable	18-22 years (n=292)	23-27 years (n=383)	28-32 years (n=332)	33-37 years (n=318)	38-42 years (n=188)	43-47 years (n=140)	48-52 years (n=109)	>52 years (n=55)
Male (%)	207 (70.9)	292 (76.2)	267 (80.4)	263 (82.7)	140 (74.5)	113 (80.7)	85 (78.0)	43 (78.2)
Female (%)	85 (29.1)	91 (23.8)	65 (19.6)	55 (17.3)	48 (25.5)	27 (19.3)	24 (22.0)	12 (21.8)
North (%)	123 (42.1)	182 (47.5)	195 (58.7)	190 (59.7)	95 (50.5)	84 (60.0)	78 (71.6)	30 (54.5)
South (%)	169 (57.9)	201 (52.5)	137 (41.3)	128 (40.3)	93 (49.5)	56 (40.0)	31 (28.4)	25 (45.5)
WB (%)	136 (46.6)	208 (54.3)	187 (56.3)	174 (54.7)	118 (62.8)	88 (62.9)	52 (47.7)	34 (61.8)
PA (%)	156 (53.4)	175 (45.7)	145 (43.7)	144 (45.3)	70 (37.2)	52 (37.1)	57 (52.3)	21 (38.2)
FTD (%)	143 (49.0)	172 (44.9)	152 (45.8)	144 (45.3)	92 (48.9)	72 (51.4)	40 (36.7)	12 (21.8)
RD (%)	89 (30.5)	113 (29.5)	82 (24.7)	100 (31.4)	58 (30.9)	30 (21.4)	46 (42.2)	30 (54.5)
BMI (kg/m^2)[a]	22.36±4.13	23.30±3.45	24.25±3.68	25.13±4.20	24.85±3.24	25.41±4.16	25.43±3.12	24.98±4.06
Number of donations[b]	2 (2-5)	2 (2-5)	2 (2-5)	2 (2-7)	2 (2-6)	2 (2-4)	2 (2-9)	5 (2-17)

[a]Mean ± SD; [b]Median (IQR). WB, whole blood donors; PA, platelets apheresis donors; BMI, body mass index; FTD, first-time donors; RD, regular donors.

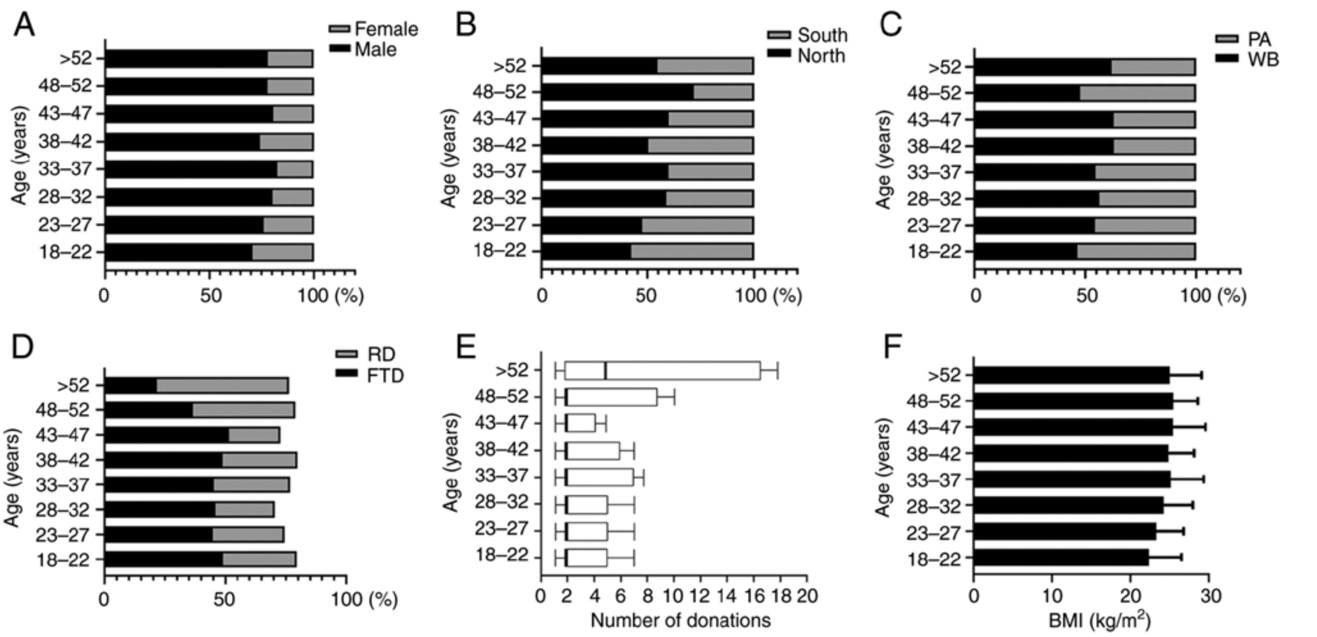

Figure 1. Demographic information stratified by age. (A) Percentage of male and female blood donors in different age groups. (B) Regional distribution of blood donors by age group. (C) Percentage of blood donation types among donors of different age groups. (D) Percentage of FTDs and RDs in different age groups. (E) Box chart of blood donation times of blood donors in different age groups. (F) BMI values of blood donors in different age groups. PA, platelets apheresis donors; WB, whole blood donors; FTD, first-time donors; RD, regular donors; BMI, body mass index.

and ferritin were transformed to a Gaussian distribution. To analyze variations according to age, gender and geography, the SDR was calculated using two-way nested ANOVA. The criterion for considering partitioning reference values was set to an SDR of >0.3 following the IFCC/C-RIDL protocol. In the present study, all SDRs were >0.3, which indicated that it was essential to establish RIs based on different ages, genders and regions (Table II).

RIs were determined by age, gender and region. Considering the inconvenience and misjudgment resulting from the excessive reference ranges for different age groups (8 groups in the present study) based on the value of SDR, a Z-test was used to confirm the necessity of stratifying the RIs and determine the cut-off age for partitioning. The Z-test results showed that there was no significant difference in the transferrin levels between different age groups. Therefore, it was not essential to establish 8 RIs based on age groups (as shown in Table I). As for the ferritin value, there was a significant difference between the older group (aged >52 years) and the younger groups (aged <52 years), while there was no significant difference between the older group (aged >52 years) and the 43-52 years old groups.

Table II. Two-way nested ANOVA for comparison of the results stratified by age, gender and region.

			Nested ANOVA		
Variable	Unit	Box-Cox (λ)	SDR_{-Age}	SDR_{-Gen}	SDR_{-Reg}
TRF	g/l	Yes (-0.5)	0.60	1.35	1.21
Fer	ng/ml	Yes (0.0)	0.77	6.59	5.69

TRF, transferrin; Fer, ferritin; SDR, standard deviation ratio. The relative magnitude of each standard deviation was expressed as SDR over SDR_{-Age}, SDR_{-Gen} and SDR_{-Reg} based on age, gender and region, respectively. For instance, $SDR_{-Age} = SD_{-Age}/SD_{-individual}$. An SDR of >0.3 was used as a guide for stratifying reference values.

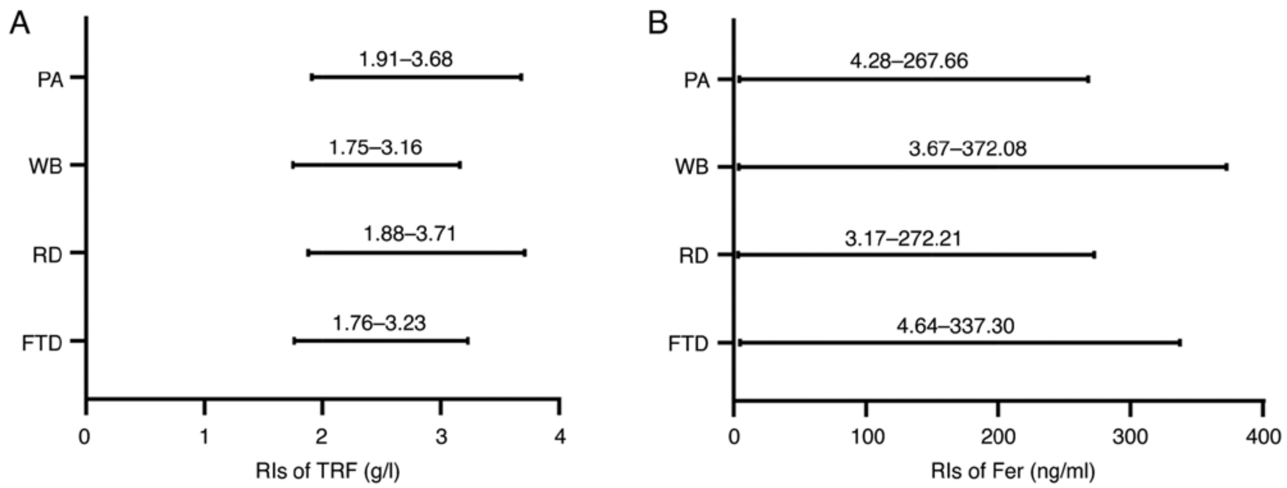

Figure 2. RIs determined by different donation frequency (FTD or RD) and donation components (PA or WB). (A) RIs of TRF determined by different donation frequency and donation components. (B) RIs of Fer determined by by different donation frequency and donation components. RIs, reference intervals; TRF, transferrin; Fer, ferritin; PA, platelets apheresis donors; WB, whole blood donors; FTD, first-time donors; RD, regular donors.

Therefore, 42 years of age was used as the cut-off age to calculate the RIs of ferritin levels. Both parametric and nonparametric methods were used to calculate RIs, and the results showed that the RIs obtained using the parametric method were wider than those obtained using the nonparametric method (Table III). After performing LAVE for secondary exclusion, 12 participants with potential iron metabolism disorders were excluded. Data from 1,805 individuals were used to establish the RIs (Table IV). The LAVE procedures generated narrower RIs for transferrin and ferritin level in the subgroups.

MRA. Blood type, BMI, whole blood or platelet apheresis donation, donation times, donation intervals, alcohol consumption and smoking were used as dependent variables to calculate the correlation coefficient using the MRA method. The ferritin level was negatively correlated with the transferrin level. As compared with whole blood donation, platelet apheresis donation was more positively correlated with transferrin, with a positive effect on serum transferrin levels. Notable changes in ferritin and transferrin levels were observed but not associated with blood donor blood type, BMI, donation times, donation interval, alcohol consumption and smoking (Table V).

RIs were determined by blood component donation and blood donation frequency. Next, RIs were established based on the donation frequency and components using the nonparametric method. As shown in Table VI and Fig. 2, regular donors had higher RIs for transferrin than first-time donors, but regular donors had a lower RI for ferritin than first-time donors. With regards to component analysis, it was observed that the transferrin RIs in whole blood donors was 1.75-3.16 g/l, while in platelet donors they were 1.91-3.68 g/l. The ferritin RIs in whole blood donors were higher than those in apheresis donors. In combination, these data suggested that the ferritin and transferrin RIs differed between first-time and regular donors, as were those between whole blood and platelet donors.

Discussion

Iron level is a vital indicator for human health (1). However, iron deficiency, a common nutritional deficiency worldwide, is a serious issue for blood donors (3,15). Thus, blood centers around the world investigated the transferrin and ferritin levels in blood donors (16-20,28). Although a large amount of data has been collected in different countries, the transferrin and ferritin levels of donors in China has not yet been fully examined. One important reason is that the RIs in China remain to be determined. Therefore, an initial objective of the project was to determine the RIs for ferritin and

Table III. Reference intervals derived by parametric and non-parametric methods for different ages, genders or regions, respectively.

			North				South			
			Male (n=813)		Female (n=164)		Male (n=597)		Female (n=243)	
Variable	Unit	Age, years	Para (LL-UL)	Nonpara (LL-UL)	Para (LL-UL)	Nonpara (LL-UL)	Para (LL-UL)	Nonpara (LL-UL)	Para (LL-UL)	Nonpara (LL-UL)
TRF	g/l	>18	1.85-3.53	1.79-3.57	1.79-3.66	1.75-3.54	1.80-3.47	1.78-3.43	1.84-3.28	1.78-3.16
Fer	ng/ml	18-42	9.24-443.68	5.48-263.61	2.76-127.51	2.48-105.36	6.01-569.98	3.67-380.33	4.05-743.82	2.70-373.55
		>42	7.74-364.27	5.90-245.86	2.76-158.14	3.02-113.32	4.20-557.97	3.00-307.69	6.17-383.45	8.76-385.63

TRF, transferrin; Fer, ferritin; Para, parametric (normal distribution method, mean ± 1.96SD); Nonpara, nonparametric (percentile method, 2.5-97.5%); LL, lower limit; UL, upper limit.

Table IV. Reference intervals calculated of data from the latent abnormal values exclusion method.

			North				South			
			Male (n=810)		Female (n=162)		Male (n=592)		Female (n=241)	
Variable	Unit	Age, years	Para (LL-UL)	Nonpara (LL-UL)	Para (LL-UL)	Nonpara (LL-UL)	Para (LL-UL)	Nonpara (LL-UL)	Para (LL-UL)	Nonpara (LL-UL)
TRF	g/l	>18	1.85-3.51	1.80-3.55	1.80-3.61	1.75-3.53	1.81-3.45	1.81-3.38	1.85-3.27	1.83-3.16
Fer	ng/ml	18-42	9.69-429.66	5.74-260.78	2.93-126.86	2.48-105.36	6.24-553.96	4.46-379.29	4.01-773.77	2.70-380.50
		>42	7.74-364.27	5.90-245.86	2.76-158.14	3.02-113.32	4.20-557.97	3.00-307.69	6.64-296.19	8.76-385.63

TRF, transferrin; Fer, ferritin; Para, parametric (normal distribution method, mean ± 1.96SD); Nonpara, nonparametric (percentile method, 2.5-97.5%); LL, lower limit; UL, upper limit.

Table V. Standardized partial regression coefficient in MRA for blood type, job type, BMI, donation type, donation times, donation interval, alcohol consumption, smoking, drugs as explanatory variables.

Variable	Blood type	BMI	Donation (WB or PA)	Donation times	Donation interval	Ethyl alcohol	Smoking	TRF	Fer
TRF	0.024	0.067	0.241[a]	0.127	-0.089	0.049	0.032	\	-0.252[a]
Fer	-0.015	0.046	-0.007	0.052	0.128	0.036	0.079	-0.283[a]	\

[a]P<0.001. BMI, body mass index; WB, whole blood donors; PA, platelets apheresis donors; TRF, transferrin; Fer, ferritin. A given explanatory variable was considered of practical importance when the absolute value of standardized partial regression coefficient was greater than 0.20.

Table VI. RIs determined by donation frequency and components donation.

Variable	Unit	Methods	FTD (LL-UL)	RD (LL-UL)	WB (LL-UL)	PA (LL-UL)
TRF	g/l	Nonpara	1.76-3.23	1.88-3.71	1.75-3.16	1.91-3.68
Fer	ng/ml	Nonpara	4.64-337.30	3.17-272.21	3.67-372.08	4.28-267.66

TRF, transferrin; Fer, ferritin; Para, parametric; Nonpara, non-transferrin level parametric. LL, lower limit; UL, upper limit; FTD, first-time donors; RD, regular donors; WB, whole blood donors; PA, platelets apheresis donors.

transferrin of Chinese blood donors. In the present study, both transferrin and ferritin level data were collected in two blood centers in China, and an RI analysis was conducted. It was also observed that apheresis platelet donation was positively correlated with serum transferrin level, as compared with whole blood donation, and that the ferritin level was negatively correlated with the transferrin level. We believe these findings are significant for the following reasons. First, the RIs of transferrin and ferritin were determined by multiple factor analysis in Chinese donors. Secondly, the collected data will help refine donor recruitment strategies in China.

Without available RIs in China, references originate from studies conducted in other countries or regions. However, due to the differences among individuals from different parts of the world, using the RIs from other countries or regions in China may not be appropriate. In fact, it was found that the RIs of transferrin and ferritin in the present study differ from those from previous studies (14,20,29). In addition, it was observed herein that iron deficiency in China is not infrequent, according to the RIs established in the present study. This study may be the most comprehensive and systematic study with the largest sample size in China that was based on the standard protocols of the CLSI and IFCC/C-RIDL. Two-way nested ANOVA and MRA were used to explore the sources of variation in the present study. In addition to the inclusion and exclusion criteria used for enrolment, the application of the LAVE method further excludes individuals with latent diseases, which is considered the appropriate approach to eliminate the effect of potential factors (26,30).

Compared with a report from Finland, the transferrin level is 1.15-2.75 mg/l in healthy adults (31). This value is lower compared with that in the present study. The reason may be due to the use of different test methods. The previous study used sandwich immunoenzymometric assay, while the present study used the turbidimetric inhibition immunoassay. With regards to the ferritin level, Chinese donors exhibited lower values (14,20). We hypothesized that ethnic/racial groups, eating habits, and/or the regional environment distinction accounted for this difference.

Donation frequency and times were considered the main factors influencing transferrin and ferritin levels (16,32). However, in the present study, it was not confirmed more frequent donations resulted in lower transferrin and ferritin levels (9,16,32). This inconsistency may be due to the difference in sample size, sample populations and/or donation policies. It should be pointed out that the minimum interval for whole blood donation was 6 months in China. Such a long interval might be a reason for the discrepancy in the results. Previous studies from other countries have suggested that prolonging the blood donation interval from 4 to 6 months can alleviate iron deficiency (7,14,33). However, the whole blood donation interval was already 180 days in China. If further extended, the shortage in blood supplies will be increased.

It was found that individuals aged >42 years exhibited lower transferrin and ferritin levels than younger donors when nonparametric methods were used. This finding was inconsistent with that of previous studies, which have suggested that adolescent donors had lower transferrin and ferritin levels (34,35). A possible reason for this is the use of different analysis methods. In addition, the elderly donors were in a lower metabolic state, with a reduced turnover of transferrin and ferritin.

Of note, it was found that apheresis platelet donation was positively correlated with serum transferrin levels compared with whole blood donation. Previous reports have often focused on serum ferritin in both types of donations (8). It was also confirmed that the ferritin level was negatively correlated with the transferrin level in blood donors, since the latter is a vital marker of iron status and its level rises with iron deficiency. It therefore seems that donation type is a key factor influencing transferrin levels.

This study had several limitations. First, it had a limited sample size; a total of 1,817 blood donor samples were collected. Secondly, the donors' transferrin and ferritin levels were not followed up after initial testing, which may have affected the adjustment of donation intervals and early detection of iron deficiency. Therefore, further large-scale data collection and longer follow-up are needed for a more comprehensive and extensive study.

In conclusion, the present results will help establish the RIs for ferritin and transferrin in Chinese blood donors. In addition, factors that influence transferrin and ferritin levels in Chinese donors were identified. Finally, these findings should help formulate improved blood donation strategies in China, protecting the health of blood donors, and contributing to the development of blood donation.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contribution

XFZ, XM and YFW performed most of the experiments and analysis. SJL, QL, ZGS, JTH, JYS, LWW and HYC were responsible for sample collection and analyzed data. XBH and XR were the research sponsors, designed the study and drafted the manuscript. XFZ and XM confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Guangzhou Blood Center [Guangzhou, China; approval no. Guangzhou Blood Center Lun (2020) No. 1]. All patients gave written informed consent before participation in this study.

Patient consent for publication

All patients gave written informed consent for publication in this study.

Competing interests

The authors declare that they have no competing interests.

References

1. Hentze MW, Muckenthaler MU and Andrews NC: Balancing acts: Molecular control of mammalian iron metabolism. Cell 117: 285-297, 2004.
2. Wang J and Pantopoulos K: Regulation of cellular iron metabolism. Biochem J 434: 365-381, 2011.
3. Camaschella C: Iron deficiency. Blood 133: 30-39, 2019.
4. Miller JL: Iron deficiency anemia: a common and curable disease. Cold Spring Harb Perspect Med 3: a011866, 2013.
5. Miller JL: Iron deficiency anemia: A common and curable disease. Cold Spring Harb Perspect Med 3: a011866, 2013.
6. Dijkstra A, van den Hurk K, Bilo HJG, Slingerland RJ and Vos MJ: Repeat whole blood donors with a ferritin level of 30 ug/L or less show functional iron depletion. Transfusion 59: 21-25, 2019.
7. Schotten N, Pasker-de Jong PC, Moretti D, Zimmermann MB, Geurts-Moespot AJ, Swinkels DW and van Kraaij MG: The donation interval of 56 days requires extension to 180 days for whole blood donors to recover from changes in iron metabolism. Blood 128: 2185-2188, 2016.
8. Duggan F, O'Sullivan K, Power JP, Healy M and Murphy WG: Serum ferritin in plateletpheresis and whole blood donors. Transfus Apher Sci 55: 159-163, 2016.
9. Rigas AS, Pedersen OB, Magnussen K, Erikstrup C and Ullum H: Iron deficiency among blood donors: Experience from the Danish Blood Donor Study and from the Copenhagen ferritin monitoring scheme. Transfus Med 29 (Suppl 1): S23-S27, 2019.
10. Di Angelantonio E, Thompson SG, Kaptoge S, Moore C, Walker M, Armitage J, Ouwehand WH, Roberts DJ and Danesh J; INTERVAL Trial Group: Efficiency and safety of varying the frequency of whole blood donation (INTERVAL): A randomised trial of 45 000 donors. Lancet 390: 2360-2371, 2017.
11. Lee SJ, Min HK, Jang JS, Lee S, Chung Y and Kim MJ: Donor protection: Iron supplementation for frequent blood donors in Korea. Transfus Apher Sci 59: 102611, 2020.
12. Garcia-Casal MN, Pasricha SR, Martinez RX, Lopez-Perez L and Peña-Rosas JP: Serum or plasma ferritin concentration as an index of iron deficiency and overload. Cochrane Database Syst Rev 5: CD011817, 2021.
13. Podmore C, Meidtner K, Schulze MB, Scott RA, Ramond A, Butterworth AS, Di Angelantonio E, Danesh J, Arriola L, Barricarte A, *et al*: Association of multiple biomarkers of iron metabolism and type 2 diabetes: The EPIC-InterAct Study. Diabetes Care 39: 572-581, 2016.
14. Vinkenoog M, van den Hurk K, van Kraaij M, van Leeuwen M and Janssen MP: First results of a ferritin-based blood donor deferral policy in the Netherlands. Transfusion 60: 1785-1792, 2020.
15. World Health Organization (WHO): Iron deficiency anaemia assessment, revention, and Control. WHO, Geneva, 2001.
16. Cable RG, Glynn SA, Kiss JE, Mast AE, Steele WR, Murphy EL, Wright DJ, Sacher RA, Gottschall JL, Tobler LH, *et al*: Iron deficiency in blood donors: The REDS-II Donor Iron Status Evaluation (RISE) study. Transfusion 52: 702-711, 2012.
17. Salvin HE, Pasricha SR, Marks DC and Speedy J: Iron deficiency in blood donors: A national cross-sectional study. Transfusion 54: 2434-2444, 2014.
18. Rigas AS, Sørensen CJ, Pedersen OB, Petersen MS, Thørner LW, Kotzé S, Sørensen E, Magnussen K, Rostgaard K, Erikstrup C and Ullum H: Predictors of iron levels in 14,737 Danish blood donors: Results from the Danish Blood Donor Study. Transfusion 54 (3 Pt 2): 789-796, 2014.
19. Baart AM, van Noord PA, Vergouwe Y, Moons KG, Swinkels DW, Wiegerinck ET, de Kort WL and Atsma F: High prevalence of subclinical iron deficiency in whole blood donors not deferred for low hemoglobin. Transfusion 53: 1670-1677, 2013.
20. Goldman M, Uzicanin S, Osmond L, Scalia V and O'Brien SF: A large national study of ferritin testing in Canadian blood donors. Transfusion 57: 564-570, 2017.
21. Horowitz G: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. 3rd edition. Clinical and Laboratory Standards Institute, Wayne, PA, 2008.
22. Ozarda Y, Ichihara K, Barth JH and Klee G; Committee on Reference Intervals and Decision Limits (C-RIDL), International Federation for Clinical Chemistry and Laboratory Medicine: Protocol and standard operating procedures for common use in a worldwide multicenter study on reference values. Clin Chem Lab Med 51: 1027-1040, 2013.

23. Ichihara K: Statistical considerations for harmonization of the global multicenter study on reference values. Clin Chim Acta 432: 108-118, 2014.
24. Molarius A, Seidell JC, Kuulasmaa K, Dobson AJ and Sans S: Smoking and relative body weight: An international perspective from the WHO MONICA Project. J Epidemiol Community Health 51: 252-260, 1997.
25. Wood AM, Kaptoge S, Butterworth AS, Willeit P, Warnakula S, Bolton T, Paige E, Paul DS, Sweeting M, Burgess S, *et al*: Risk thresholds for alcohol consumption: Combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. Lancet 391: 1513-1523, 2018.
26. Ichihara K and Boyd JC; IFCC Committee on Reference Intervals and Decision Limits (C-RIDL): An appraisal of statistical procedures used in derivation of reference intervals. Clin Chem Lab Med 48: 1537-1551, 2010.
27. Zeng X, Fang L, Peng Y, Zhang Y, Li X, Wang Z, Zhang B, Cao Q and Hu X: A multicenter reference interval study of thromboelastography in the Chinese adult population. Thromb Res 195: 180-186, 2020.
28. van den Berg K, Swanevelder R, Ingram C, Lawrie D, Glencross DK, Hilton C and Nieuwoudt M: The iron status of South African blood donors: Balancing donor safety and blood demand. Transfusion 59: 232-241, 2019.
29. World Health Organization (WHO): WHO guideline on use of ferritin concentrations to assess iron status in individuals and populations. WHO, Geneva, 2020.
30. Ichihara K, Ceriotti F, Tam TH, Sueyoshi S, Poon PM, Thong ML, Higashiuesato Y, Wang X, Kataoka H, Matsubara A, *et al*: The Asian project for collaborative derivation of reference intervals: (1) strategy and major results of standardized analytes. Clin Chem Lab Med 51: 1429-1442, 2013.
31. Suominen P, Punnonen K, Rajamäki A and Irjala K: Serum transferrin receptor and transferrin receptor-ferritin index identify healthy subjects with subclinical iron deficits. Blood 92: 2934-2939, 1998.
32. Mittal R, Marwaha N, Basu S, Mohan H and Ravi Kumar A: Evaluation of iron stores in blood donors by serum ferritin. Indian J Med Res 124: 641-646, 2006.
33. Spekman MLC, Ramondt S and Sweegers MG: Whole blood donor behavior and availability after deferral: Consequences of a new ferritin monitoring policy. Transfusion 61: 1112-1121, 2021.
34. Patel EU, White JL, Bloch EM, Grabowski MK, Gehrie EA, Lokhandwala PM, Brunker PAR, Goel R, Shaz BH, Ness PM and Tobian AAR: Association of blood donation with iron deficiency among adolescent and adult females in the United States: A nationally representative study. Transfusion 59: 1723-1733, 2019.
35. Vassallo RR, Hilton JF, Bravo MD, Vittinghoff E, Custer B and Kamel H: Recovery of iron stores after adolescents donate blood. Pediatrics 146: e20193316, 2020.