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Research Article

Correlation Between Soluble Transferrin Receptor Versus Haemoglobin Concentration, Transferrin Saturation and Serum Ferritin Levels Following Repeated Blood Donations in Nigeria

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Abstract

Background: Soluble serum transferrin receptor (sTfR) is an index of erythropoietic activity and it is a useful test to diagnose iron deficiency anaemia. In this study, the levels of sTfR and other iron-related parameters and the association with each other were evaluated. **Materials and Methods:** One hundred and eighty-four subjects comprising five groups were recruited into this study, that is, 35 (19.0%) of control group, 32 (17.4%) of first, 35 (19.0%) of second, 41 (22.3%) of third and 41 (22.3%) of fourth time blood donors, within the age range of 18-49 years. Iron-related parameters namely; haemoglobin concentration (Hb), transferrin saturation (TS), serum iron (SI) and total iron binding capacity (TIBC) were determined using the photometric method, while serum ferritin level (SF) and serum transferrin receptor level (STfR) were determined using enzyme immunoassay (ELISA). **Results:** STfR correlated negatively with haemoglobin concentration ($p < 0.05$), transferrin saturation, serum iron and serum ferritin levels ($p < 0.01$), while sTfR correlated positively with TIBC in 184 male blood donors ($p < 0.01$). Based on the frequency of blood donation, STfR correlated negatively with Hb [fourth time donors], with SI [third and fourth ($p < 0.01$) time donors], with TS [fourth and third time donors], with SF [third ($p < 0.05$) and fourth ($p < 0.05$) time donors] and positively with TIBC. **Conclusion:** These results suggested that the level of sTfR may be a useful indicator of iron deficiency in blood donors and should be considered with another test as a criterion especially in donors donating three and four times within a year.

Key words: Serum transferrin receptor, correlation, blood donors, serum ferritin, transferrin saturation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Transferrin receptor (TfR) is a glycoprotein that transverse the plasma membrane of most mammalian cells but localizes mainly on developing erythrocytes¹. This receptor is of two types (TfR1 and TfR2) and is involved in conveying iron into these cells in response to their iron status or concentration². The concentration of this receptor varies with iron demand and development of cells during erythropoiesis³. Therefore, assaying for this receptor is crucial for determining the cellular iron concentration and erythropoietic activity. The concentration of this receptor in serum is influenced by conditions such as iron deficiency anaemia (IDA) and anaemia of chronic disease (ACD)⁴.

Skikne *et al.*⁵ and Flowers *et al.*⁶ found that there is no difference in sTfR values for healthy adult men and women and no correlation of STfR with age of their enrolled participants. Blood donation is a process in which a person voluntarily donates his or her blood which is provided to another needy person. Every transfusion service has detailed regulations which slightly modify this general statement and which are designed either for the protection of the donor or the recipient⁷. Voluntary blood donation remains a challenge in developing countries as blood and blood products are only sourced from close relatives and colleagues who have been certified to be at low risk for transfusion-transmissible infections (TTIs)⁸. An anaemic person cannot donate blood. To protect against this possibility, haemoglobin evaluation is required for every blood donor on each donation. In Nigeria, the minimum values for haemoglobin concentration are set at 12.5 g dL⁻¹ for all blood donors^{9,10}, while in European countries the cut-off level is 12.5 g dL⁻¹ for women and 13.5 g dL⁻¹ for men¹¹. Iron deficiency is a common clinical problem that, in most instances, is relatively simple to diagnose using such conventional laboratory tests of iron status such as serum iron, total iron binding capacity, transferrin saturation and ferritin. However, these tests are considerably influenced by acute phase responses, making it difficult to distinguish between iron deficiency anaemia and anaemia that accompanies infection, inflammation or malignancy¹². The latter is commonly termed anaemia of chronic disease. The anaemia of chronic disease is the most common cause of anaemia in hospitalized patients¹³. This form of anaemia typically develops in patients suffering from chronic inflammatory disorders that involve activation of cellular immunity. The serum ferritin level varies with iron stores, while TfR is assumed to reflect the degree of tissue iron supply¹⁴. It is now appreciated that depletion of iron-dependent tissue enzymes occurs in concert with a decrease in haemoglobin

production¹⁵. Serum sTfR and sTfR/ferritin ratio are useful parameters in hypochromic microcytic anaemia to improve the diagnosis of iron deficiency, particularly when associated with chronic inflammation^{16,17}. Serum transferrin receptor (sTfR) is better than serum ferritin (SF) as a screening test for iron deficiency. Unlike serum values of iron and ferritin, serum transferrin receptor levels are not influenced by elevation in cytokine synthesis in patients with ACD¹⁸. In addition, serum transferrin receptor when compared to serum ferritin can be used to distinguish iron deficiency anaemia from anaemia of chronic disease¹⁹. Serum transferrin receptor (sTfR) measurements are probably most valuable in a setting where most donors are repeated donors²⁰. Certain professionals are sceptical concerning the frequency of blood donations with subsequent intake of iron supplements and have recommended that adult males donate twice, while their female counterparts donate once a year²¹. There is an evident clinical need for non-invasive and sensitive means for the detection of iron deficiency and the serum transferrin receptor (TfR) level has been introduced as a promising new tool for the diagnosis of iron depletion²². A lot of other biochemical iron-related parameters notably, serum iron, total iron binding capacity, transferrin saturation and serum ferritin have been studied in frequent blood donors in Calabar and Nigeria as a whole, except serum transferrin receptor were much work as not been done. Therefore, current study aim was to assess haemoglobin concentration, serum iron, total iron binding capacity, transferrin saturation and serum ferritin and to check their association with serum transferrin receptor.

MATERIALS AND METHODS

The study was conducted between August 2012 to December 2012 at the University of Calabar Teaching Hospital (UCTH), Blood Donor Bay. Approval was given by the Health Research Ethical Committee (HREC) of the Hospital before the collection of samples commenced. Permission was gotten using written informed consent from the donors. Bio-data along with the medical history of each participant was obtained using questionnaire before blood donation. This was a cross-sectional study with a total of 184 subjects comprising of five groups, that is, 35 (19.0%) control group (donors donating for the first time), 32 (17.4%) of first (donors donating for the second time), 35 (19.0%) of second (donors donating for the third time), 41 (22.3%) of third (donors donating for the fourth time) and 41 (22.3%) of fourth (donors donating for the fifth time) time blood donors. There were 450 donors who came to the UCTH bleeding bay during the four months of sample collection in 2011 and they all donated

blood within the period of two months. After the selection of donors using questionnaire, 184 male blood donor's samples were analyzed. The donors were within the range of 18-49 years of age. The inclusion criteria were donors that had packed cell volume of $>0.400 \text{ L L}^{-1}$; those that donated blood in a previous period of fewer than two months; seronegative for HIV serotypes 1 and 2, hepatitis B and C and *Trepanoma pallidum* (Syphilis). The exclusion criteria were those on an iron supplement or had gone through major surgery in the past three years and those that had a history of blood transfusion in the past two years.

Collection of sample: Blood samples were collected from each male participant after blood donation and it was dispersed into 2 different sample bottles (EDTA and plain sterile bottles). EDTA bottle was used for sample collection for haemoglobin concentration using complete automated cell counter (ERMA INC. Tokyo PCE-210, 5.10 versions). Blood in the sterile iron-free bottle was used for estimation of biochemical iron-related parameters. It was allowed to clot, dislodged and later spun at 4000 g for 10 min and the serum from each subject was dispersed into two separate sterile plain bottles. One aliquot was utilised to analyse serum iron and binding capacity of iron using photometric/colorimetric technique (TECO diagnostic USA, Lot number 1592), while transferrin saturation was derived from iron concentration divided by the binding capacity of iron value and multiplied by 100 (expressed as a percentage). The second bottle was utilized for serum ferritin and serum transferrin receptor assays using ELISA (Catalog number: BC-1025 and BioVendor-Laboratori medicinaa.s. Cat. number: RD194011100 Czech Republic). Both sera were kept at -20°C until ready for analysis.

Data analysis: Data generated from laboratory assays were appropriately analysed for means, standard deviation and Pearson's correlation using SPSS version 20 (SPSS Inc., Chicago, IL, USA). Results were presented as an average $\pm$ standard error of the mean. Two-sided $p = 0.05$ was considered statistically significant for Pearson's correlation when assessing the association between sTfR and SI, Hb, TIBC and SF.

RESULTS

The age distribution of participants and their frequency of blood donation was revealed in Fig. 1. This figure indicated that majority of the donors were in the age ranges of 18-25 years (45.7%) and 26-35 years (47.3%), while ≥ 36 years (7.1%) were very few.

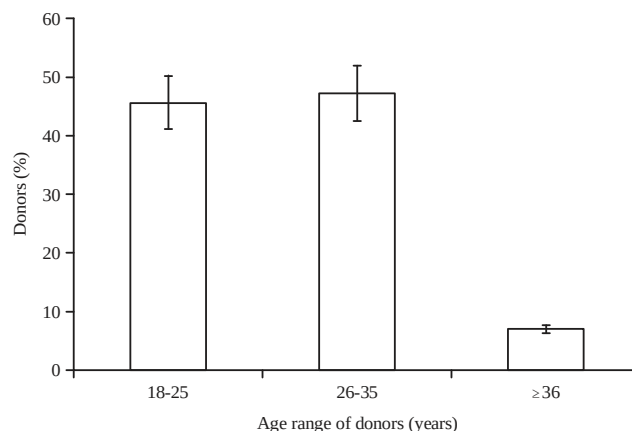


Fig. 1: Age range of 184 age male blood donors and their respective percentages

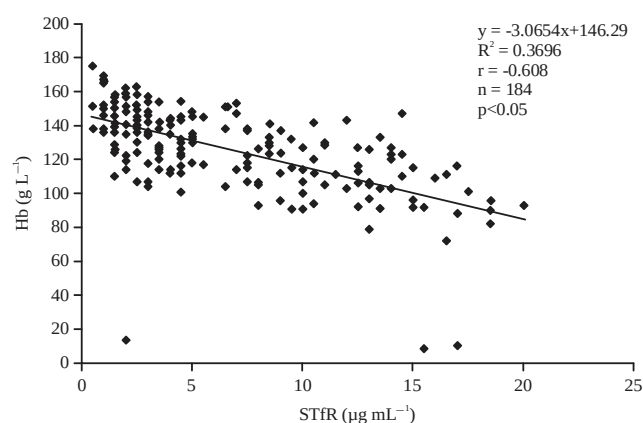


Fig. 2: Correlation graph between STfR and Hb of 184 blood donors

A negative significant associations which is moderate between sTfR and Hb ($r = 0.608$, $p < 0.05$), weak between sTfR and SF ($r = 0.309$, $p < 0.05$), moderate between sTfR and SI ($r = 0.539$) as well as between sTfR and TS ($r = 0.596$) in blood donors was shown in Fig. 2-4 and 6. A moderate positive significant association which was observed between sTfR and TIBC ($r = 0.537$, $p < 0.05$) in all participating blood donors was shown in Fig. 5. Generally, in this study, it was observed that as Hb, SF and TS reduced, sTfR increased, while as TIBC was raised, sTfR was raised as well.

Haemoglobin concentration (Hb) correlated significantly with serum transferrin receptor (sTfR) among repeated blood donors as shown in Table 1. It showed a significant negative correlation (r) between Hb and sTfR in all participant groups [zero time donors ($r = -0.231$, $p < 0.05$), first time donors ($r = -0.382$, $p < 0.05$), second time donors ($r = -0.204$, $p < 0.05$), third time donors ($r = -0.199$, $p < 0.05$), fourth time ($r = -0.568$, $p < 0.01$)]. So, as Hb concentration reduces, sTfR increases.

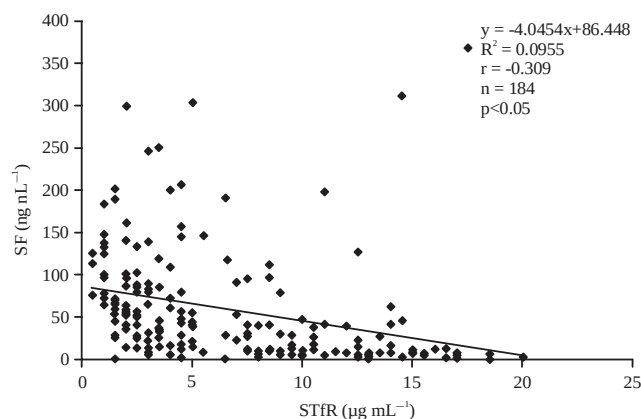


Fig. 3: Correlation graph between STfR and SF of 184 blood donors

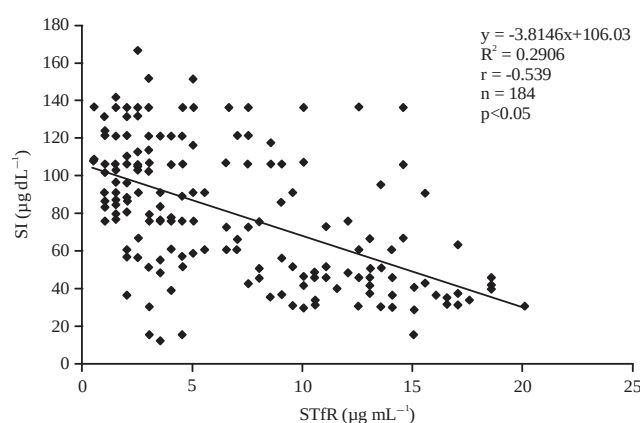


Fig. 6: Correlation graph between STfR and SI of 184 blood donors

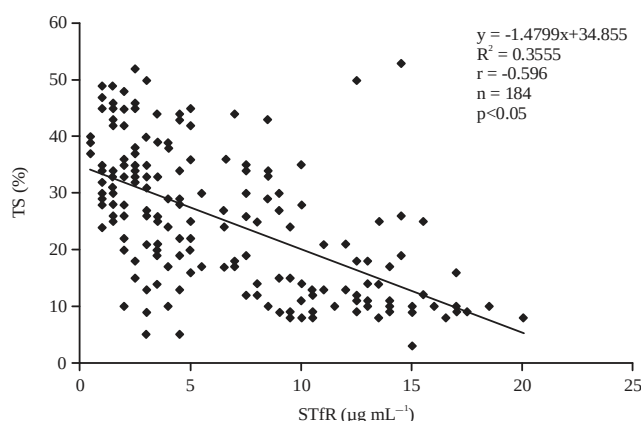


Fig. 4: Correlation graph between STfR and TS of 184 blood donors

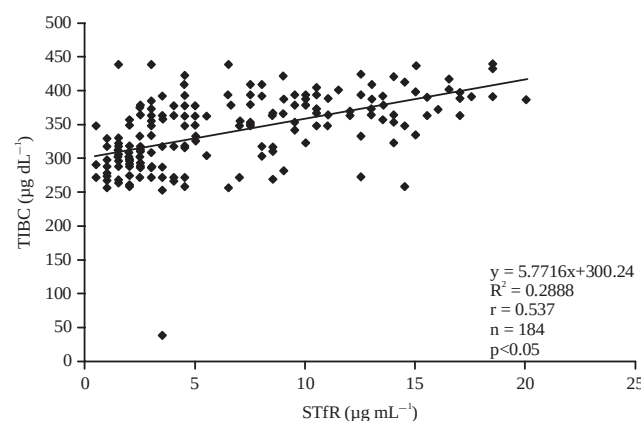


Fig. 5: Correlation graph between STfR and TIBC of 184 blood donors

The association between SI and sTfR of blood donors was shown in Table 2. It shows no significant correlation (r) in

Table 1: Correlation coefficient (r) of Hb vs sTfR in blood donors

Serial donations	Coefficient of correlation (r)	p-value
Control group (n = 35)	-0.231	*
First-time donors (n = 32)	-0.382	*
Second-time donors (n = 35)	-0.204	*
Third-time donors (n = 41)	-0.199	*
Fourth-time donors (n = 41)	-0.568	**

*p<0.05, **p<0.01

Table 2: Correlation coefficient (r) of SI vs sTfR in blood donors

Serial donations	Coefficient of correlation (r)	p-value
Control group (n = 35)	0.032	NS
First-time donors (n = 32)	0.000	NS
Second-time donors (n = 35)	-0.349	*
Third-time donors (n = 41)	-0.505	**
Fourth-time donors (n = 41)	-0.405	**

*p<0.05, **p<0.01, NS: Not significant

Table 3: Correlation coefficient (r) of TIBC vs sTfR in blood donors

Serial donations	Coefficient of correlation (r)	p-value
Control group (n = 35)	0.203	*
First time donors (n = 32)	0.209	*
Second time donors (n = 35)	0.361	*
Third time donors (n = 41)	0.499	**
Fourth time donors (n = 41)	0.170	*

*p<0.05, **p<0.01

control group and first time blood donors, but weak negative correlation with second (r = -0.349, p<0.05), third (r = -0.505, p<0.01) and fourth (r = -0.405, p<0.01) time blood donors. It was observed that as the value for SI decreased, that of sTfR increased.

The association between TIBC and sTfR of blood donors and non-donors was shown in Table 3. It showed a negligible positive significant correlation (r) in zero (r = 0.203, p<0.05), first (r = 0.209, p<0.05), second (r = 0.361, p<0.05) and fourth (r = 0.170, p<0.05) time blood donors, except in third (r = 0.499, p<0.01) time blood donors that showed a moderate positive significant correlation. So,

Table 4: Correlation coefficient (r) of TS vs sTfR in blood donors

Serial donations	Coefficient of correlation (r)	p-value
Control group (n = 35)	-0.071	NS
First-time donors (n = 32)	-0.095	NS
Second-time donors (n = 35)	-0.428	**
Third-time donors (n = 41)	-0.609	**
Fourth-time donors (n = 41)	-0.419	**

**p<0.01, NS: Not significant

Table 5: Correlation coefficient (r) of SF vs sTfR in blood donors

Serial donations	Coefficient of correlation (r)	p-value
Control group (n = 35)	-0.078	NS
First time donors (n = 32)	-0.237	*
Second time donors (n = 35)	-0.197	*
Third time donors (n = 41)	-0.245	*
Fourth time donors (n = 41)	-0.362	*

*p<0.05, NS: Not significant

as TIBC increased in control group, first, second, third and fourth time donors, sTfR also increased.

The association between TS and sTfR of blood donors and non-donors was shown in Table 4. It showed no significant correlation (r) between these variables in control group and first time blood donors, ($p>0.05$) but a significant negative correlation which is weak in second ($r = -0.428$, $p<0.05$) and fourth ($r = -0.419$, $p<0.05$) and moderate in third ($r = -0.609$, $p<0.05$) time blood donor. It was observed that as TS reduced sTfR increased.

The association between SF and sTfR blood donors and non-donors was shown in Table 5. It showed no significant correlation (r) between these variables in control group ($p>0.05$), but negligible negative correlation in first ($r = -0.237$, $p<0.05$), second ($r = -0.197$, $p<0.05$), third ($r = -0.245$, $p<0.05$) and fourth ($r = -0.362$, $p<0.05$) time blood donors. It was observed that as SF reduced, sTfR increased.

DISCUSSION

Soluble serum transferrin receptor (sTfR) is an index of erythropoietic activity²³. It is a very reliable test since it is not affected by inflammation²⁴. Serum transferrin receptor increases when there is iron deficiency. Several parameters that may affect iron deficiency, notably, Hb, SI, TIBC, TS and SF have therefore been correlated with sTfR, a marker of erythropoietic activity with a view to finding out whether the parameters affect erythropoietic activity (Table 1-5). All the biochemical iron-related parameters with the exception of TIBC correlated negatively with sTfR. This indicates that as the values for Hb, SI, TS and SF reduced, sTfR increased and vice versa. In general, HB, SI, TS and SF decreased with each successive blood donation while TIBC increased. Since sTfR increased significantly as Hb, SI, TS and SF reduced, the results

of this study were indicative of an increase in erythropoietic activity in the bone marrow with each successive blood donation. This significant relationship means that with time, iron stores in the body may be depleted. These observations are in accordance with that of Yousefinejad *et al.*²⁵, who reported significant associations between levels of Hb, SF, SI, TS and TIBC and the number of times for blood donation. In addition, previous studies²⁶⁻²⁸ observed that increased frequency of blood donation decreased serum ferritin level.

Total iron binding capacity (TIBC) in general correlated positively with serum transferrin receptor (sTfR) (Table 3). This signifies that as sTfR (an indicator of erythropoietin activity of the bone marrow) increased, the TIBC also increased. So repeated blood donation which stimulates erythropoietin activity also improves iron binding capacity. The increased capacity to bind iron following repeated blood donation may be stimulated by progressively reduced stores of iron and so, TIBC increased in the bid to acquire enough iron for the body in the situation of dwindling supplies of iron. This has not been previously reported among repeated blood donors in Nigeria, but this current study has showed the importance of carrying out some of the iron-related parameters, preferably sTfR as an indicator for being selected for blood donation. These findings are in line with previous studies^{29,27} who observed that blood donors had significantly higher TIBC compared to their non-donor counterparts.

The percentages of the total number of subjects at various times of blood donation were also similar. They ranged from 17.4-22.3% of the total number of 184 at each time of blood donation (Fig. 1). The similarity in percentages of blood donors at various times of blood donation is good since it ruled out bias when comparing groups. However, 45.7% of the total blood donors were in the 18-25 year group and 47.3% were in the 26-35 year group. Only a few (7.1%) were above 36 years old. The reason for younger subjects, that is, persons below 36 years donating blood is not certain but these could be attributed to unemployment in Nigeria because statistics show that 40 million Nigerians are unemployed and are mainly youths³⁰. The findings of this present study are not in agreement with that of Shaz *et al.*³¹, who reported a peak percentage of blood units donated by participants within 40-49 years when compared to other age groups in the United States. The disparity in these observations could be variation both in living standards and literacy.

CONCLUSION AND FUTURE RECOMMENDATION

Soluble serum transferrin receptor (sTfR) an index of erythropoietic activity which signals iron deficiency showed a

significant negative correlation with Hb, SI, TS and SF but a positive correlation with TIBC. Therefore, measuring the donor's sample for the sensitive serum transferrin receptor assay is preferable in order to assess the iron stores. There is also a great need to educate blood donors about iron supplementation and there should be a 3 months interval between blood donations with proper documentation of each successive blood donation.

SIGNIFICANCE STATEMENT

To the best of our knowledge, this is the first comparative study of iron-related parameters with reference to sTfR performed on blood donors in this region. Apart from serving as a baseline for further research, this study has provided a better means of assessing the suitability of prospective blood donors and how these donors can know when to fortify their iron stores based on the knowledge of their iron status at an earlier stage of iron deficiency.

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