

## Serum Soluble Transferrin Receptor and Transferrin Levels among Regular Blood Donors

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### Abstract

**Background:** The study evaluated the effects of regular blood donation on serum transferrin and soluble transferrin receptor levels at Wenchi Methodist Hospital. **Methods:** This was a hospital-based cross-sectional study conducted at the Medical Laboratory Department of the Wenchi Methodist Hospital in the Bono Region of Ghana. A total of eighty-nine (89) venous blood samples from apparently healthy blood donors were analyzed. Complete blood count parameters were analyzed using an automated haematology analyzer and serum transferrin and transferrin receptor using ELISA. The data were analyzed using SPSS version 22.0. **Results:** Haemoglobin ( $p<0.001$ ) and HCT ( $p=0.004$ ) were significantly lower among the regular blood donors compared with the first-time donors. Regular blood donors had relatively higher serum transferrin ( $p<0.001$ ) and soluble transferrin receptor levels ( $p<0.001$ ). A negative correlation was observed between Hb and serum transferrin ( $r=-0.552$ ,  $p<0.001$ ), as well as Hb and serum soluble transferrin receptor ( $r=-0.552$ ,  $p<0.001$ ). Remunerated donors had lower Hb ( $p=0.001$ ) and HCT% ( $p=0.001$ ) but a higher transferrin receptor ( $p=0.041$ ) than non-remunerated donors. **Conclusion:** Regular blood donors had relatively lower erythrocyte parameters but higher serum transferrin and soluble transferrin receptors, indicating a possible reduction in serum iron and iron stores. Moderate negative correlations exist between Hb and both transferrin and soluble transferrin receptors. Again, remunerated donors had lower erythrocyte parameters but higher transferrin and soluble transferrin receptors than non-remunerated donors. Periodic assessment of iron parameters among regular blood donors is recommended. A future longitudinal study to assess the entire iron profile of regular blood donors is recommended.

**Keywords:** Regular Blood Donors; Serum Transferrin; Serum Soluble Transferrin Receptors; ELISA.

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## 1. Introduction

Regular blood donation is an essential component of global health care and serves as an effective practice to ensure the availability of blood for immediate blood transfusion, especially when a patient's haemoglobin (Hb) levels fall below 7-8 g/dL [1]. Even though globally about one hundred million units of blood are donated each year, there is still a high demand for blood and blood products in low-income countries [2]. The increased demand for blood transfusions, particularly in sub-Saharan Africa, puts blood donor collection facilities under significant pressure to expand their donor pool to be able to stock blood banks for emergency interventions. The World Health Organization (WHO) has suggested that blood donation at a rate of 1/100 people is adequate to cover the transfusion demands of any community, but Sub-Saharan African countries fall short of meeting this critical requirement, covering just 41.5% of yearly transfusion demands [3]. Access to blood varies significantly across low- and high-income nations. In high-income countries, there are 31.5 blood donations made for per 1,000 people. This contrasts with 16.4 donations per 1000 people in upper-middle-income countries, 6.6 donations per 1000 people in lower-middle-income countries, and 5.0 donations per 1000 people in low-income countries [3].

Donating one unit of blood (450 mL) results in a loss of about 250 mg of iron, which is about 30% and 80% of the average male and female body iron reserves, respectively [4]. Since every 1 mL of blood donated leads to a loss of 0.5 mg of iron, individuals who donate whole blood on a regular basis are at risk of iron deficiency [5]. Blood donation may therefore be a potential contributor to the development of a negative iron balance in people with borderline iron storage in countries such as Ghana, where anaemia is a significant public health problem [6]. Varying nations have different selection requirements for blood donors. For instance, in Ghana and Taiwan, male blood donors must have a haemoglobin value of at least 13.0 g/dL and at least 12.0 g/dL for female blood donors prior to blood donation, but a minimum donor Hb of 13.5 g/dL for males and 12.5 g/dL for females is required in the United Kingdom [7]. Haemoglobin concentration measurement is an established part of donor selection tests to safeguard against collecting blood from an anaemic individual, but this is insensitive to iron deficiency during its early stages as iron deficiency anaemia is the last stage of iron deficiency pathogenesis [5]. In developing countries like Ghana, where iron deficiency is widespread, relying solely on prospective donors' haemoglobin levels for donor recruitment may be risky [8].

Although blood donors are warmly praised for their noble gesture of donating blood to save lives, little to no attention is paid to their health situation. The health of the receiver is often prioritized over the health of the donor. Ferritin, the most widely used biochemical test for assessing the status of iron, is less sensitive because it is an acute-phase protein and affected by chronic inflammation. Transferrin and soluble transferrin receptors are better early indicators of iron deficiency and can provide better information on the status of iron [9].

Despite the sensitive values of transferrin and soluble transferrin receptor over routine haemoglobin assessment, no known study has been conducted in Ghana to evaluate the effects of regular blood donation on serum transferrin and soluble transferrin receptor levels. Thus, this study evaluated the effects of regular blood donation on serum transferrin and soluble transferrin receptor levels at Wenchi Methodist Hospital. Findings from this study will aid in the development of policies to protect prospective blood donors, reduce donor deferral rates, and ensure the supply of blood and blood products for life-saving medical interventions.

## 2. Materials and Methods

### 2.1. Study Design/Study Site

This was a cross-sectional study from January to April, 2022 at the Methodist Hospital, Wenchi, a Christian faith-based municipal hospital in the Bono Region of Ghana. The 238-bed capacity hospital serves as a referral hub for 20 public and private healthcare facilities in the Wenchi municipality. Wenchi municipality is located in the eastern part of Bono Region, and it lies within latitudes of 7° 30' South, 7° 15' North and longitudes 2° 17' West and 1° 55' East. According to the 2021 Population and Housing Census, Wenchi Municipal has a population of approximately 124,758 people [10].

### 2.2. Study Population and Sample Size

The study recruited 89 blood donors: 61 regular donors who had donated blood at least twice within a year and 28 first-time donors as controls, based on Cochran's formulae, as has been previously described [11]. All blood donors filled out the universal donor recruitment questionnaire and met the predonation selection criteria of weight  $\geq 50$  kg, haemoglobin concentration ( $\geq 12.5$  g/dL), body temperature ( $37^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$ ) and being non-reactive for transfusion-transmitted infections (HIV, hepatitis B and C, and syphilis). Donors with low haemoglobin levels, known liver diseases, or communicable diseases, as indicated by the WHO, were excluded from the study [12].

### 2.3. Blood Sample Collection and Processing

Five milliliters (5 mL) of blood samples were aseptically collected into serum separator (2 mL) and K<sub>3</sub>EDTA (3 mL) tubes. The blood in the K<sub>3</sub>EDTA tube was gently inverted 8–10 times to ensure adequate mixing of the blood with the anticoagulant. The K<sub>3</sub>EDTA blood was used for complete blood count analysis using a three-part haemoglobin auto-

analyzer. The gel tube blood was allowed to clot and centrifuged at 3,000 rpm/5 min to obtain the serum. The serum was separated into Eppendorf tubes and stored at -80 °C. The stored plasma was subsequently used for the determination of transferrin and soluble transferrin receptor levels using a sandwich Enzyme-Linked Immunosorbent Assay (ELISA).

### 2.4. Complete Blood Count Measurement

A complete blood count (CBC) was performed on each day of sample collection. A fully automated three-part haematology analyzer (Mindray BC3000 Plus, China) was used for the estimation of the CBC parameters. The haematology analyzer employs electrical impedance, flow cytometry, and colorimetric principles. The blood cells were measured by creating an electrical field around a calibrated micro-aperture through which the cells flow after being diluted in an electrolytic diluent. Red blood cell parameters [haemoglobin (Hb), absolute red blood cell count (RBC), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and red cell distribution width-coefficient of variation (RDW-CV)], white blood cell parameters: [total white blood cell count (TWBC), absolute lymphocytes, absolute granulocytes (Neutrophils) and MID or MIXED (Comprising Monocytes, Eosinophils, and Basophils)] and platelet parameters: [Platelet count and mean platelet volume (MPV)] were computed.

### 2.5. Measurement of Serum Transferrin and Soluble Transferrin Receptors using ELISA

Transferrin and transferrin receptor levels were assayed by the sandwich ELISA method using commercially prepared ELISA kits (Biobase, China). All procedures were carried out according to the manufacturer’s instructions for each analyte. The ELISA plates were washed using an automated ELISA washer (Bio-Rad PW 40, France), while the plasma concentrations of the analytes were determined using a microplate ELISA reader (Mindray MR-96A, China).

### 2.6. Data Analysis

The data generated were analyzed using IBM Statistical Package for the Social Sciences (SPSS) software version 22.0 (Armonk, New York, USA). Normality was tested with one-sample Kolmogorov-Smirnov tests and Shapiro-Wilk normality tests. Parametric and non-parametric data were presented as means ± standard deviation, and medians (25<sup>th</sup>-75<sup>th</sup> percentiles), respectively. The chi-square or Fisher's exact test was used appropriately to determine the association between bivariate categorical variables. Parametric data were compared appropriately with Students’ T test and One-Way ANOVA, while non-parametric data were compared appropriately using the Mann-Whitney U test and Kruskal-Wallis test. The Spearman rank correlation test was used to determine the association between two numerical variables. A  $p < 0.05$  was considered statistically significant.

## 3. Results

A total of 89 participants, comprising 61 regular blood donors and 28 first-time donors as controls, were recruited for the study. The majority of the participants (39/43.8%) were between the ages of 25 and 34, with only 6 (6.7%) older than 44 years. About one-third of the subjects had attained Junior High School while 7 (7.9%) never attended school. Most (61/68.5%) of the participants were remunerated donors (Table 1).

**Table 1. Demographic Characteristics of the Study Subjects**

Variables	Category	Frequency (%)
Gender	Male	86 (96.6)
	Female	3 (3.4)
Age group (years)	<25 years	31 (34.8)
	25-34 years	39 (43.8)
	34-44 years	13 (14.6)
	>44 years	6 (6.7)
Marital Status	Single	69 (77.5)
	Married	20 (22.5)
Educational Status	None	7 (7.9)
	Primary	6 (6.7)
	JHS	33 (37.1)
	SHS	29 (32.6)
	Tertiary	14 (15.7)
Donor Type	Remunerated	61 (68.5)
	Non-remunerated	28 (31.5)

Data are represented in frequencies with percentages in parentheses.

Table 2 shows the percentage distribution of blood donors based on whether they were first-time donors or regular donors. Twenty-eight (28/31.4%) of participants were first-time donors, with (61/ 68.5%) being regular donors. The majority (12/42.9%) of the first-time donors were under the age of 25, while that of frequent blood donors were between the ages of 25 and 34. Most (8/28.6%) of first-time donors had completed tertiary-level education, while (27/44.3%) of repeat donors had attained Junior high-level education. Individuals who had finished basic school and those with no education at all had the lowest frequency for both first-time and repeat donors (Table 2).

**Table 2. Participants’ Demographics between First-Time and Regular Blood Donors**

Variables	Categories	Blood Donors		P-value
		First-Time (%) (N=28)	Regular (%) (N=61)	
Gender	Male	28 (100)	58 (95.1)	0.128
	Female	-	3 (4.9)	
Ages (years)	<25	12 (42.9)	19 (31.1)	0.146
	25-34	10 (35.7)	29 (47.5)	
	35-44	6 (21.4)	7 (11.5)	
	>44	0 (0.0)	6 (9.8)	
Marital Status	Single	23 (82.1)	46 (75.4)	0.590
	Married	5 (17.9)	15 (24.6)	
Educational Status	None	4 (14.3)	3 (4.9)	<b>0.007</b>
	Primary	4 (14.3)	2 (3.3)	
	JHS	6 (21.4)	27 (44.3)	
	SHS	6 (21.4)	23 (37.7)	
	Tertiary	8 (28.6)	6 (9.8)	

N=Number of participants. Pearson-Chi Square test and Fishers’ exact test were used to compare marital status, and gender respectively, and One-Way ANOVA was used to compare multivariate variables. Data are represented in frequencies with percentages in parentheses. p<0.05 was considered statistically significant.

Regular blood donors had relatively lower Hb, g/dL: 13.33 (13.07-14.40) vs 14.75 (14.15-15.35),  $p<0.001$ ;  $RBC \times 10^{12}/L$ :  $4.65 \pm 0.51$  vs  $5.11 \pm 0.55$ ,  $p<0.001$  and HCT:  $41.38 \pm 4.59$  vs  $45 \pm 6.75$ ,  $p=0.004$  compared to the first-time donors. But, MCV, WBC, MCH, RDW-CV, Absolute Lymphocyte, Absolute Granulocytes, Platelet, and MPV were not different between first-time and regular blood donors.

**Table 3. Complete Blood Count Parameters between First-Time and Regular Blood Donors**

Variables	Blood Donors			P-value
	First-time (N=28)	Regular (N=61)	Total	
Hb (g/dL)	14.75 (14.15-15.35)	13.33 (13.07-14.40)	13.80 (13.10-14.85)	< <b>0.001</b>
RBC $\times 10^{12}/L$	$5.11 \pm 0.55$	$4.65 \pm 0.51$	$4.89 \pm 0.53$	< <b>0.001</b>
HCT (%)	$45.0 \pm 6.75$	$41.38 \pm 4.59$	$43.02 \pm 5.34$	<b>0.004</b>
MCV (fL)	$89.89 \pm 5.02$	$88.45 \pm 7.47$	$88.90 \pm 6.8$	0.357
MCH (pg)	$29.07 \pm 1.77$	$28.49 \pm 2.84$	$28.67 \pm 2.56$	0.327
MCHC (g/dL)	$32.36 \pm 0.99$	$32.23 \pm 1.0$	$32.27 \pm 0.99$	0.580
RDW-CV (%)	14.55 (14.05-15.75)	14.50 (13.80-15.80)	14.50 (13.90-15.80)	0.937
WBC $\times 10^9/L$	6.05 (5.10-6.85)	5.60 (4.80-6.50)	5.80 (4.80-6.60)	0.052
Abs. Lymph $\times 10^9/L$	$2.54 \pm 0.89$	$2.46 \pm 0.73$	$2.48 \pm 0.78$	0.628
Abs. GRA $\times 10^9/L$	3.05 (2.20-3.50)	2.60 (2.10-3.20)	2.70 (2.10-3.20)	0.153
Platelet $\times 10^9/L$	$232.75 \pm 79.27$	$229.07 \pm 64.31$	$230.22 \pm 68.92$	0.816
MPV (fL)	$8.10 \pm 0.8$	$8.2 \pm 0.9$	$8.18 \pm 0.87$	0.590

Hb=Hemoglobin, HCT=Haematocrit, MCV=Mean Cell Volume MCH=Mean Corpuscular Hemoglobin, MCHC=Mean Corpuscular Hemoglobin Concentration, RDW-CV=Red Cell Distribution Width-Coefficient of Variation, WBC=White Blood Cell, Abs. GRA= Absolute Granulocyte, Abs. Lymph= Absolute Lymphocyte, MPV=Mean Platelet Volume, L=litre, g/dL=Grams per decilitre, fL=Femtolitre, pg=Picogram. Parametric data (presented in means  $\pm$  standard deviation) was generated by Student T-test, and non-parametric data (presented in medians (25th-75th percentiles) generated by Mann-Whitney U-test, p<0.05 was considered statistically significant.

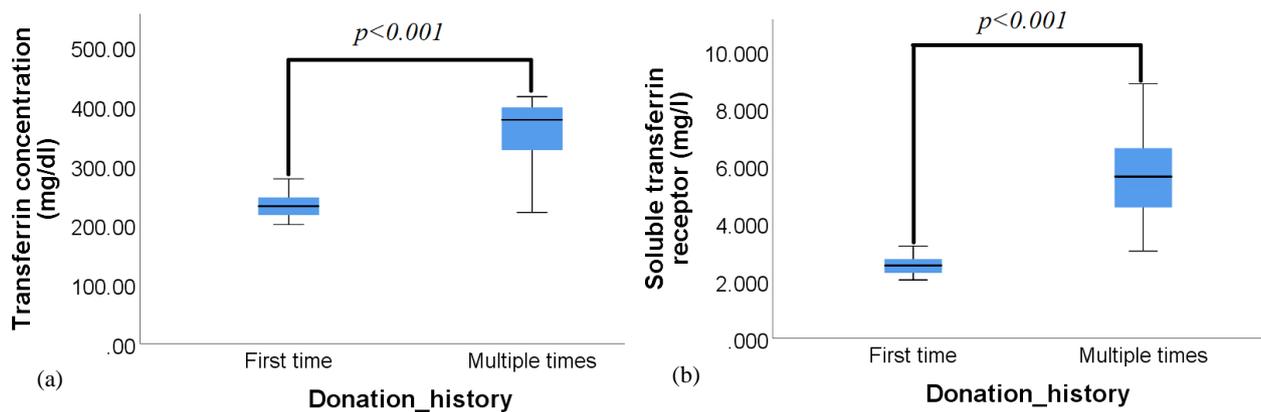
Hb, RBC and HCT decreased across the number of donation intervals. Participants who had donated blood at least seven times had lower red blood cell parameters compared to their counterparts who had donated few times. Other blood cell parameters were not different within the groups (Table 4).

**Table 4. Complete Blood Count Parameters of the Regular Blood Donors Stratified by the Number of Blood Donations- Hb=Hemoglobin, HCT=Haematocrit, MCV=Mean Cell Volume MCH=Mean Corpuscular Hemoglobin**

Variables	Number of Blood Donations				P-value	Significant Pairs
	2-3 Times <sup>a</sup>	4-5 Times <sup>b</sup>	6-7 Times <sup>c</sup>	>7 Times <sup>d</sup>		
RBC ×10 <sup>12</sup> /L	4.89 ± 0.54	4.87 ± 0.35	4.49 ± 0.49	4.38 ± 0.51	<b>0.007</b>	<b>a&amp;d (p=0.030), b&amp;d (p=0.028), a&amp;c (p=0.011), a&amp;d (p=0.008).</b>
Hb (g/dL)	14.50(13.50-15.20)	13.75(13.10-14.50)	13.20(12.10-13.80)	13.20(13.00-13.32)	<b>0.017</b>	
HCT (%)	43.20 ± 5.24	43.08 ± 2.96	40.43 ± 4.72	39.08 ± 4.34	<b>0.029</b>	
MCV (fL)	88.69 ± 8.01	88.69 ± 5.59	89.27 ± 9.48	87.06 ± 6.67	0.866	
MCH (pg)	28.59 ± 3.01	28.77 ± 2.12	28.35 ± 3.61	28.26 ± 2.63	0.959	
MCHC (g/dL)	32.26±0.87	32.47±0.84	31.76±1.00	32.48±1.14	0.123	
RDW-CV %	15.00(14.20-15.80)	14.15(13.75-14.75)	14.80(14.10-16.20)	14.50(13.70-15.80)	0.407	
Platelet ×10 <sup>9</sup> /L	227(169-277)	246(220-274)	208(202-242)	205(182-239)	0.193	
MPV (fL)	7.92±0.66	8.29 ± 1.05	8.87±1.01	7.90 ± 0.36	0.759	
WBC ×10 <sup>9</sup> /L	5.80(4.80-7.40)	5.30(4.95-5.85)	5.70(4.90-6.40)	4.80(4.35-6.35)	0.758	
GRA count ×10 <sup>9</sup> /L	2.90(2.00-3.30)	2.70(2.15-3.15)	2.60(2.50-3.20)	2.20(1.95-2.95)	0.647	
Monocyte ×10 <sup>9</sup> /L	0.60(0.40-0.70)	0.40(0.35-0.55)	0.60(0.50-0.70)	0.40(0.30-0.65)	0.193	
Lymphocyte count ×10 <sup>9</sup> /L	2.40(2.10-2.90)	2.60(1.95-2.90)	2.10(1.80-2.60)	2.00(1.90-2.35)	0.958	

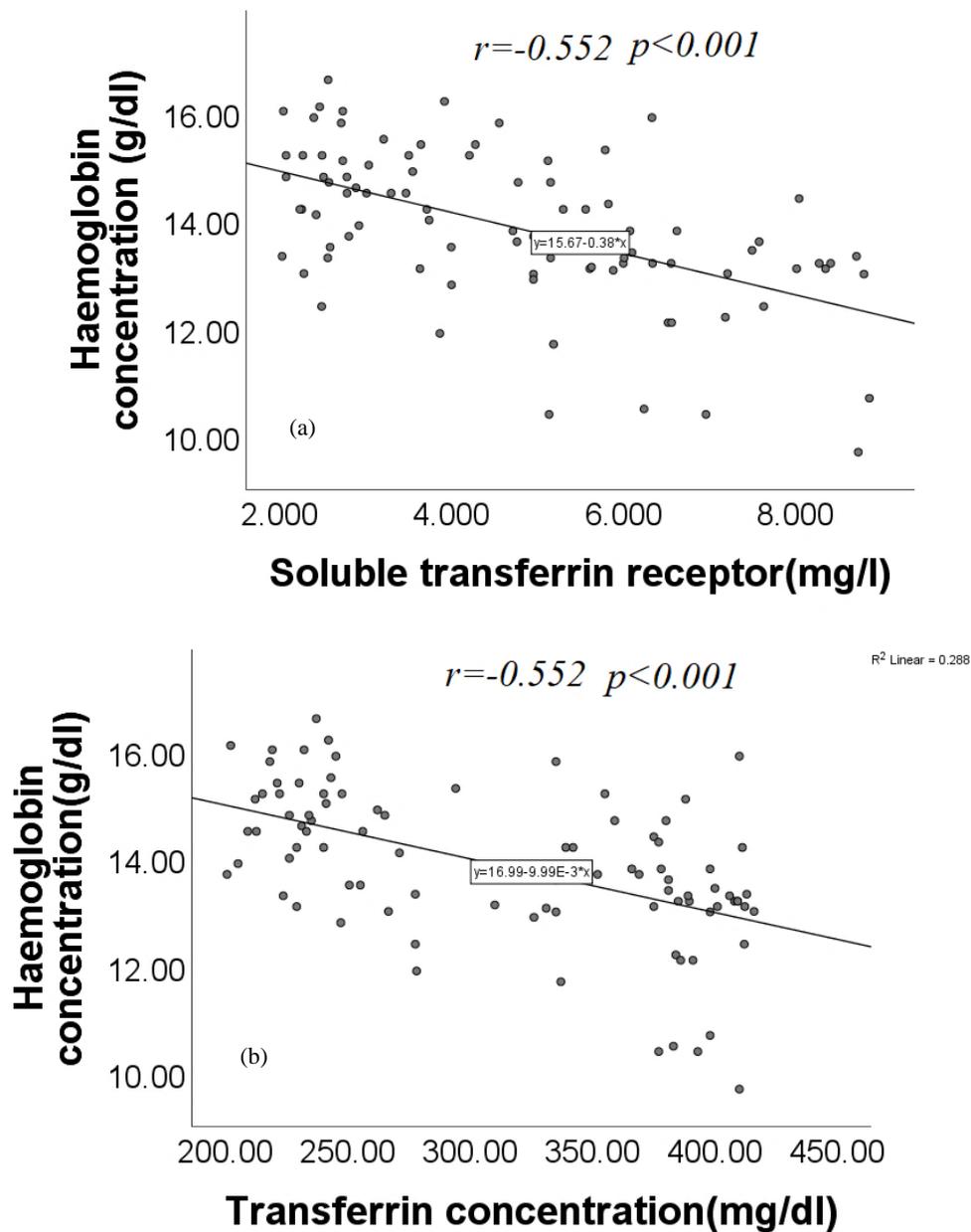
MCHC=Mean Corpuscular Hemoglobin Concentration, RDW-CV=Red Cell Distribution Width-Coefficient of Variation, WBC=White Blood Cell, GRA=Granulocyte, MPV=Mean Platelet Volume. Parametric data (presented in means ± standard deviation) were generated by One-Way ANOVA and Non-Parametric data presented in medians (25th-75th percentiles) were generated by Kruskal-Wallis Test. p<0.05 was considered statistically significant.

Figure 1 shows the serum soluble transferrin receptor (Figure 1-a) and transferrin concentrations (Figure 1-b) between first-time and regular blood donors. The median serum soluble transferrin receptor and soluble transferrin level were higher among the regular donors compared with their counterparts who had donated blood for the first time: Serum transferrin receptor (mg/L): [5.63(4.56-6.62) vs 2.54(2.28-2.76), p<0.001]; Transferrin (mg/dL): [377.00 (326.00-389.00) vs 231.50 (216.50-246.25), p<0.001].



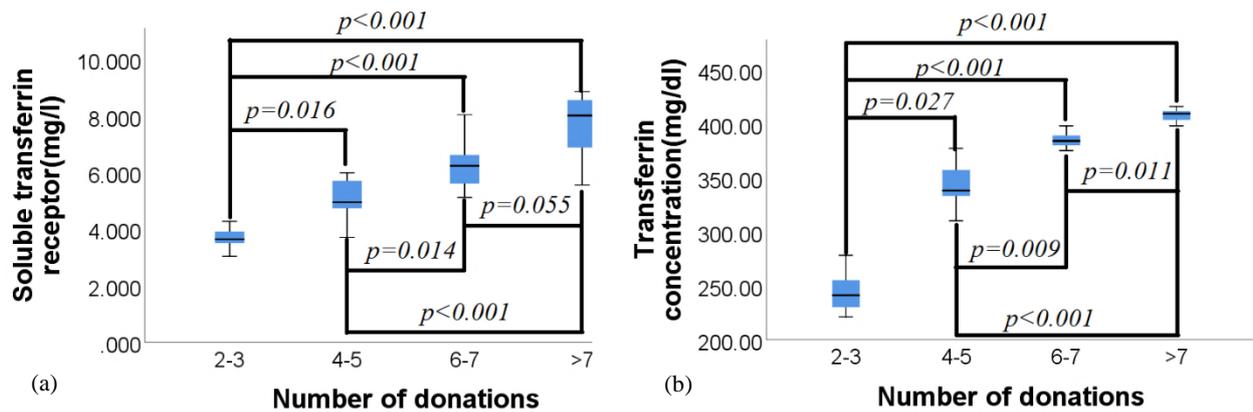
**Figure 1. Serum Transferrin and Soluble Transferrin Receptor Concentration among First-Time vs Regular Blood Donors. mg/dL=milligram per decilitre, mg/L= milligram per litre. Data were generated by Mann-Whitney U-Test and p<0.05 was considered statistically significant.**

Figure 2 illustrates the correlation between haemoglobin concentration (Hb) and serum transferrin (Figure 2-a) and soluble transferrin receptor concentration (Figure 2-b) among the blood donors. A moderately negative correlation was observed between Hb and serum transferrin (r=-0.552, p<0.001), as well as Hb and soluble transferrin receptor concentrations (r =-0.552 p<0.001) among the blood donors.



**Figure 2. Correlation between Hemoglobin Concentration, and Serum Transferrin and Soluble Transferrin Receptor Concentration among the Study Participants:  $r$ =Correlation coefficient, mg/dL=milligram per decilitre, mg/L= milligram per litre, g/dL=Grams per decilitre. Correlation was assessed by Spearman rank correlation test and  $p < 0.05$  was considered statistically significant.**

Figure 3 demonstrates the relationship between serum soluble transferrin receptor (Figure 3a) and transferrin concentration (Figure 3b) and the number of times regular donors donated blood. A statistically significant difference was found between serum soluble transferrin receptor concentration and the number of donations among regular blood donors: [2-3 times: 3.65 (3.48-3.96) vs >7 times: 8.02 (6.56-8.71) mg/L,  $p < 0.001$ ], [2-3 times: 3.65 (3.48-3.96) vs 6-7 times: 6.24 (5.38-6.79) mg/L,  $p < 0.001$ ], [2-3 times: 3.65 (3.48-3.96) vs 4-5 times: 4.95 (4.73-5.75)mg/L,  $p < 0.016$ ], [>7 times: 8.02 (6.56-8.71) vs 4-5 times: 4.95 (4.73-5.75) mg/L,  $p < 0.001$ ], [4-5 times: 4.95 (4.73-5.75), vs 6-7 times: 6.24 (5.38-6.79) mg/L,  $p = 0.014$ ]. Also, there was a significant difference between transferrin concentration and number of donations among regular donors [2-3 times: 241.00 (229.50-255.50) mg/dL vs >7 times: 409.30 (401.00-412.00) mg/dL,  $p < 0.001$ ], [2-3 times: 241.00 (229.50-255.50) mg/dL vs 6-7 times: 384.00 (332.00-358.00) mg/dL,  $p < 0.001$ ], [2-3 times: 241.00 (229.50-255.50) mg/dL vs 4-5 times: 338.00 (332.00-358.00) mg/dL,  $p = 0.027$ ], [4-5 times: 338.00 (332.00-358.00) mg/dL vs >7 times: 409.30 (401.00-412.00) mg/dL,  $p < 0.001$ ], [4-5 times: 338.00 (332.00-358.00) mg/dl vs 6-7 times: 384.00 (332.00-358.00) mg/dL,  $p = 0.009$ ], [6-7 times: 384.00 (332.00-358.00) mg/dL vs >7 times: 409.30 (401.00-412.00) mg/dL,  $p = 0.011$ ].



**Figure 3.** Association between Serum Soluble Transferrin Receptor (a) and Transferrin Concentration (b), and Number of Blood Donations among the Regular Blood Donors. mg/dL=milligram per decilitre, mg/L= milligram per litre, difference was assessed by Kruskal Wallis.  $p<0.05$  was considered statistically significant.

Table 5 shows information about the haematological parameters of the participants and their blood donation type. There was significance difference in some parameters of the complete blood count between remunerated and non-remunerated donors: Hb, g/dL: 13.50 (13.10-14.50) vs 14.75 (13.45-15.65),  $p=0.001$ ; HCT%:  $41.20\pm 5.32$  vs  $45.37\pm 5.14$ ,  $p=0.001$ ; MCH, pg:  $28.18\pm 2.57$  vs  $29.74\pm 2.19$ ,  $p=0.007$ ; MCV, fL:  $87.72\pm 6.91$  vs  $91.46\pm 5.87$ ,  $p=0.015$ ; MCHC, g/dL:  $32.12\pm 0.94$  vs  $32.58\pm 1.04$ ,  $p=0.044$ ; and serum transferrin receptor, mg/L: 4.95 (2.91-6.54) vs 3.59 (2.56-5.50),  $p=0.041$ . However, WBC, RDW-CV, Absolute Lymphocyte, Absolute Granulocytes, Platelet, MPV and serum transferrin did not differ between remunerated and non-remunerated blood donors.

**Table 5.** Complete Blood Count, Serum Transferrin and Soluble Transferrin Receptor Concentrations of the Participants Stratified by their Blood Donor Types

Variables	Blood Donor type		P-value
	Remunerated	Non-remunerated	
Hb (g/dL)	13.50 (13.01-14.50)	14.75 (13.45-15.65)	<b>0.001</b>
RBC $\times 10^{12}/L$	$4.72\pm 0.51$	$4.94\pm 0.64$	<b>0.043</b>
HCT (%)	$41.20\pm 5.32$	$45.37\pm 5.14$	<b>0.001</b>
MCV (fL)	$87.72\pm 6.91$	$91.46\pm 5.87$	<b>0.015</b>
MCH (pg)	$28.18\pm 2.57$	$29.74\pm 2.19$	<b>0.007</b>
MCHC (g/dL)	$32.12\pm 0.94$	$32.58\pm 1.04$	<b>0.044</b>
RDW-CV (%)	14.70 (14.00-16.20)	14.20 (13.90-14.75)	0.087
WBC $\times 10^9/L$	5.80 (4.90-6.70)	5.45 (4.60-6.30)	0.205
Absolute Lymphocyte $\times 10^9/L$	$2.53\pm 0.82$	$2.38\pm 0.70$	0.379
Abs. GRA $\times 10^9/L$	2.90 (2.20-3.20)	2.50 (1.75-3.10)	0.203
Platelet $\times 10^9/L$	$235.16\pm 69.19$	$219.46\pm 68.33$	0.321
MPV (fL)	$8.17\pm 0.9$	$8.2\pm 0.79$	0.952
Transferrin (mg/dL)	342.00(237.00-390.25)	264.25(235.50-376.50)	0.150
Transferrin receptor (mg/L)	4.95 (2.91-6.54)	3.59 (2.56-5.50)	<b>0.041</b>

Hb=Haemoglobin, HCT=Haematocrit, MCV=Mean Cell Volume MCH=Mean Corpuscular Haemoglobin, MCHC=Mean Corpuscular Haemoglobin Concentration, RDW-CV=Red Cell Distribution Width-Coefficient of Variation, WBC=White Blood Cell, GRA=Granulocyte, MPV=Mean Platelet Volume. Parametric data (presented in means  $\pm$  standard deviation) were generated by Independent T test, and non-Parametric data (presented in medians (25th-75th percentile)) were generated by Mann-Whitney U Test.  $p<0.05$  was considered statistically significant.

#### 4. Discussion

Regular blood donors are at higher risk of developing negative iron balance due to an estimated loss of 250 mg of iron per unit (~500 mL) of whole blood donated [4]. This study evaluated serum levels of transferrin and soluble transferrin receptors among regular blood donors.

In this study, there were predominantly more male blood donors than female blood donors, which is consistent with other studies in Ghana. In the middle belt of Ghana, Nkansah et al. [13] noted a female-to-male ratio of 1:4.8 among blood donors at Nkenkaasu District Hospital; and in the Tamale Teaching Hospital's Blood Donation Center, where more than 70% of blood donors were men in a study by Mohammed & Essel [14]. This result is also consistent with previous studies in Nigeria [15], India [5], and Brazil [16]. This finding could be related to the mistaken cultural perception that only men are healthy enough to donate blood, perhaps because they do not menstruate. In Ghana, women

are considered vulnerable and protected from undertaking risky activities. Blood donation is perceived as a risky procedure reserved for men.

The majority of repeat donors in this study were between the ages of 25 and 34, which is consistent with the findings from the Mohammed & Essel study [14]. Again, close to 80 percent of first-time donors were under 35 years old, and this is in agreement with a previous study conducted at the Southern Area Blood Centre in Ghana's Greater Accra Region [17]. Also, most of the blood donors in this study, including both first-time and recurring donors, had finished junior high school. These findings could be attributed to the fact that most of these donors (JHS leavers and under 35 years old) are unemployed and motivated by the small reward given to them by the blood bank facilities.

The relatively lower red blood cell parameters among regular blood donors observed in this study are consistent with earlier findings [18–21]. This finding may be associated with the tendency of repeat donors to become anaemic after three to four donations. However, this contradicts the findings from other studies elsewhere [5, 22, 23].

The Sultana et al. [23] study found RDW to be a better marker for early identification of iron insufficiency, but the present study did not identify any changes in RDW among the regular and first-time donors. One possible explanation for the variation may be that even though RDW is increased in rapidly developing iron insufficiency, it does not increase in persistent subclinical deficit [20]. Moreover, this study found lower concentrations of Hb, HCT, MCV, MCH, and MCHC in remunerated donors than in non-remunerated donors. The reduction in red cell parameters observed in remunerated donors is consistent with the findings from the study by Benedict et al. [24]. This could be attributed to the uncontrolled frequency of donations by remunerated donors, as they often visit various donation sites in an effort to enhance their remuneration. Also, the compensated donors in this study had higher median soluble transferrin receptor concentrations than the non-remunerated donors, possibly due to the substantial reduction in haemoglobin concentration. Various health jurisdictions require standard blood donation intervals as a safety practice to protect the donor. Failure to adhere to the standard practice poses a health threat to the donor as the body fails to replenish the loss of blood and its constituents, which eventually leads to iron depletion and other complications.

The relatively higher levels of serum transferrin and soluble transferrin receptors among regular donors compared to first-time donors are in accordance with previous studies by Adediran et al. [25], Mahida et al. [21], and Eisenstein & Ross [26], who suggested that inducing an iron deficit could enhance the effectiveness of transferrin secretion. This is because the substantial loss of red blood cells from the body through regular blood donation could elicit the release of more soluble transferrin and transferrin receptors in response to the associated iron deficiency.

The present study also found a moderately negative correlation between haemoglobin concentration and serum transferrin, as well as soluble transferrin receptors. These findings are consistent with those from an earlier study, which observed a significant negative correlation between transferrin receptors and haemoglobin [27]. This discovery may be explained by the ongoing synthesis of transferrin receptors in response to the associated iron deficiency or decrease in haemoglobin concentration from regular donation. Age, gender, marital status, or level of education did not show a significant association with both serum transferrin and haemoglobin. These findings contradict an earlier study in India, where haemoglobin levels were higher in males than females and also showed a positive correlation with age [28]. While about ninety-seven percent of the study participants in this study were males, the Namita [28] study included 55.9% adolescent males, and this could have accounted for the differences in the findings. The study was limited by its inability to assess the entire iron profile of the study participants.

## 5. Conclusion

Regular blood donors had relatively lower red blood cell parameters, and this may contribute to the development of iron deficiency anaemia. Serum transferrin and soluble transferrin receptors are higher among regular blood donors, indicating a possible reduction in serum iron/stores. Moderately negative correlations exist between haemoglobin concentration and both serum transferrin and soluble transferrin receptors among the blood donors. Again, remunerated donors had lower red cell parameters but higher transferrin and soluble transferrin receptors than non-remunerated donors. Strict regulation of blood donation practices locally and adherence to standard practices to protect prospective blood donors are recommended. Also, a further study is recommended to holistically assess the iron profile of regular blood donors. Regular blood donors with suspected iron deficiency may be provided with an iron supplement.

## 6. Declarations

### 6.1. Author Contributions

Conceptualization, S.K.A., C.N.1, V.K., D.E.N., B.O.O., C.A.A., K.M., and S.B.B.; laboratory works, S.K.A., K.M., V.K., D.E.N., C.A.A., C.N.1, B.O.O., Y.Q., C.N.2, C.A.E.W., and G.A.; formal analysis, C.N.1, K.M., S.K.A., V.K., D.S. and F.O-B.; resources, C.N.1, S.K.A., Y.Q., C.A.D., G.A., K.M., D.S., V.K., C.A.A., B.O.O., F.O-B., and S.B.B.; data curation, D.E.N., V.K., B.O.O., C.A.A., and Y.Q.; writing—original draft preparation, S.K.A., C.N.1, C.N.2, K.M., V.K., Y.Q., F.E.C., F.O-B., G.A., D.S., C.A.D., S.B.B. and S.D.; writing—review and editing, S.K.A., F.E.C., C.N.1, K.M., S.B.B., G.A., F.O-B., D.S., C.A.D., D.E.N., S.D., B.O.O., C.A.A., C.A.E.W., V.K., Y.Q., C.N.2, G.A., and S.D. All authors have read and agreed to the published version of the manuscript.

## 6.2. Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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The authors received no financial support for the research, authorship, and/or publication of this article.

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## 6.5. Ethical Approval and Institutional Review Board Statement

Ethical approval was sought from the Institutional Review Board of the University for Development Studies, Tamale, Ghana (UDS/RB/024/22). Permission was obtained from the management of Methodist Hospital, Wenchi.

## 6.6. Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

## 6.7. Declaration of Competing Interest

The authors declare that there is no conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

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