

# Assessment of iron status in regular, replacement and voluntary male blood donors of Peshawar Khyber Pakhtoonkhwa

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**Assessment of iron status in regular, replacement and voluntary male blood donors of  
Peshawar Khyber Pakhtoonkhwa**

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## Abstract:

### Objective

The objectives of this study were to identify iron deficiency in regular, replacement and voluntary male blood donors and to determine that serum ferritin levels are helpful in detecting pre-clinical iron deficiency, hence should be added in donor selection criteria.

### Methodology

A cross-sectional descriptive study was conducted. Male blood donors (n= 152) were divided into regular and non- regular (1<sup>st</sup> time, replacement and voluntary ) groups on the basis of number of donations given in the past. Qualitative C- reactive protein analysis was done to exclude any coexisting inflammatory illness. To identify red cell indices complete blood count was performed and to determine body iron status, quantitative determination of serum ferritin levels were performed.

### Results

Seventeen (73.9%) regular blood donors while 13 (10.1%) of the non-regular blood donors developed iron deficiency. Among non-regular blood donors, 09% 1<sup>st</sup> time donors, 9.3% of replacement donors and 15.8% of the voluntary donors had iron deficiency. Overall, iron deficiency anemia was recorded in 19.7% of the whole study sample of 152. Statistically significant inverse relationship existed between number of donations and serum ferritin levels. ( $r = -0.193$ ,  $p 0.017$ ). Also a weak positive relationship between time since last donation (months) and serum ferritin levels (pearson  $r = 0.109$ ,  $p 0.18$ )

### Conclusion

Repeated whole blood donations increase the chances of iron deficiency. Pre-donation serum ferritin analysis would help to prevent iron deficiency in regular blood donors. Prolongation of

inter-donation interval or provision of iron supplements may allow frequent donation without developing iron deficiency.

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**Key words:**

**Regular blood donors, serum ferritin, iron deficiency**

## Introduction:

Blood is donated worldwide so it is our ethical duty to prevent the blood donors from harm.<sup>1</sup> Iron deficiency in first time and repeat blood donors is challenging in transfusion medicine.<sup>2</sup> The influence of blood donation on the body's iron status has been a subject of interest since 1970's<sup>3</sup>. Blood can be donated safely, at a minimum of eight weeks interval but at the risk of developing iron deficiency with repeated donations<sup>4, 5</sup>. A strong association has been observed between donation frequency, time since last donation and iron stores<sup>4</sup>. The Hb reaches the pre-donation level by 30 to 50 days in first time donors.<sup>1</sup> There is no major risk associated with blood donation however, studies have documented that regular blood donors are iron depleted<sup>6, 7</sup>. Inadequate dietary iron intake cannot resume iron balance in blood donors<sup>2</sup>. Eligibility criteria for blood donation is a cut-off Hb value of 12.5g/dL<sup>8</sup>. Use of Hb as a screening tool to exclude anaemic donors sometimes fails to identify iron deficient donors whose Hb is above the cutoff limits<sup>9</sup>.

The reticulocytes, Hb content and percentage of hypochromic red cells are very helpful in detecting functional iron deficiency but they have not been validated in the past.<sup>6</sup> Each 1 mL of blood contains 0.5 mg of iron roughly, hence donation of one unit of whole blood removes 4-10 % ( approximately 250 mg ) of the body iron and with resulting fall in Hb after 3 days<sup>8, 10</sup>. It takes about 150 days to replenish 220-320 mg of iron lost by donation. Hence repeated donations cause further iron depletion<sup>1, 11</sup>.

Hemoglobin levels may not correlate significantly with the underlying iron status of the donor and is not a sensitive test to rule out the early stages in the development of iron deficiency<sup>6, 12</sup>. For precise assessment of the body's iron level and diagnosis of iron deficiency, different blood parameters have been recommended<sup>9, 13, 14</sup>.

Serum ferritin (Fe) level is considered most consistent test for the assessment of iron status of the body. Low levels of serum ferritin occur <sup>5</sup> when there is a reduction in the iron stores of the body<sup>6</sup>.<sup>14</sup> However, ferritin is an acute phase reactant as well. Therefore a high level of serum ferritin may show a false result unless presence of any infectious or inflammatory condition is not ruled out<sup>15, 16</sup>.

Keeping in mind, the widespread incidence <sup>37</sup> of iron deficiency in blood donors, this study <sup>44</sup> has been designed to identify preclinical iron deficiency in regular, replacement and voluntary male blood donors. To estimate the distribution of regular, replacement and voluntary male blood donors among the population of Peshawar-KPK and to determine whether the widely accepted inter-donation interval should be increased for donor safety regarding iron status.

## Mathodology

<sup>27</sup> A cross-sectional descriptive study was conducted in the Department of Pathology, North West General Hospital (NWGH) and Research Centre Peshawar, Department of Haematology, Hayatabad Medical Complex (HMC) Peshawar and <sup>1</sup> Institute of Basic Medical Sciences (IBMS), Khyber Medical University Peshawar from 1<sup>st</sup> December, 2012 to 30<sup>th</sup> May, 2013. Ethical approval was taken from the institutional research ethical committee. Venous blood samples from 152 male blood donors were collected from the fore mentioned Tertiary Care Hospitals and analyzed after taking an informed consent.

<sup>12</sup> Non-probability purposive sampling technique was used. The total sample population was <sup>21</sup> divided into four main categories, according to the number of blood donations and frequency of donations, **Group A:** 1<sup>st</sup> time blood donors (they had not given any previous blood donation) **Group B:** Regular donors (they had donated blood 3-4 times previously, in last 12 months), **Group C:** Replacement donor (they were patient's relatives or friends who had given  $\geq 1$  donations in last 12 months), **Group D:** Voluntary donor <sup>9</sup> (those who donated blood without remuneration or any family relation). <sup>4</sup> The blood donors, included in this study were healthy males aged between 17-60 years, weight more than 50kg with hemoglobin (Hb) level  $> 13.0\text{g/dl}$  while the patients with raised CRP and with the history of hospitalization in the past 6 months were excluded.

<sup>36</sup> Blood samples were taken in two vacutainer tubes, Ethylene Diamine Tetra Acetic Acid (EDTA) and a plain tube with gel. Complete blood count was performed on the same day on Automated Hematology Analyzer (Sysmex XS-1000i, Sysmex Corporation, America). To exclude any acute condition or inflammatory condition, qualitative CRP analysis was done using CRP-latex test

based on slide agglutination method using the kit ( SPINREACT, Spain). Only CRP negative blood samples were included for further analysis. Quantitative determination of circulating ferritin concentrations in human serum was done by an immunoenzymometric sequential assay by using Microplate Chemiluminescence immunoassay (CLIA) (Acculite CLIA Microwells, Monobind Incorporation, USA).

<sup>4</sup> In this study iron stores were considered normal for serum ferritin level above 30µg/L, values between 15-30µg/L were reduced and < 15 were iron deficiency <sup>12, 17</sup> . For diagnosing <sup>4</sup> iron deficiency and anemia in male blood donors, various cut-off values were set. The cut-off criteria indicating anemia in adult males were Hemoglobin < 13g/dl <sup>18</sup>, Hematocrit < 39 % , Red cell distribution width > 14% <sup>19</sup>, Serum ferritin ≤ 15µg/L<sup>17</sup>, Microcytosis as mean corpuscular volume of MCV < 80 fl <sup>17</sup>.

<sup>17</sup> The data was analyzed using Statistical Package for Social Sciences (SPSS) version 20. <sup>2</sup> Comparison between groups was done using chi-square test. For quantitative variables, mean ± standard deviation was designed. Pearson's correlation coefficient was applied on different iron parameters to measure the degree of linear relationship between variables.



# RESULTS

The study was conducted on 152 male blood donors of which 129 (84.9%) were non regular blood donors while 23 (15.1%) were regular blood donors. Among 129 non regular blood donors, 51.9% were 1<sup>st</sup> time blood donors, 33.3% were replacement donors and 14.7% were voluntary blood donors. The baseline characteristics of the included sample are detailed in Table No 1:

Table 1: Baseline characteristics of Blood donors

Donor type		Age in years (range)	No of Donations (range)
Non regular donors	1 <sup>st</sup> time donor (n = 67)	17-55	0
	Replacement donor (n = 43)	18-45	1-5
	Voluntary donor (n = 19)	19-48	1-8
Regular Donors	Regular donor (n = 23)	18-47	4-20
TOTAL (n = 152)		17-55	1-20

From all the included donors, a sample of blood was obtained and tested in the laboratory of the institute of basic medical sciences KMU, for certain hematological and biochemical parameters. Table 2 elaborates various parameters tested on donor blood sample before the blood donations is executed.

The mean MCV of the whole study sample was  $81.3 \pm 4.4$  fl, mean MCH was  $27.4 \pm 2.2$  pg, mean RDW was  $10.8 \pm 0.8\%$ , mean serum ferritin was  $72.5 \pm 71.6$  ug/L, mean platelets count was  $269.3 \pm 59.5 \times 10^9/L$ , mean Hemoglobin was  $14.7 \pm 0.8$  gm/dl and mean hematocrit was  $43.6 \pm 2.6\%$ .

On applying one way ANOVA at 5% significance level, we observed a statistically significant difference between the donor groups and MCV ( $p 0.02$ ), RDW ( $p 0.005$ ) and serum ferritin ( $p 0.001$ ). See Table 2.

**Table 2: Comparison of biochemical and hematological parameters among blood donor groups**

Parameter	Non Regular Donors			Regular donors (n = 23)	One way ANOVA (P value)
	1 <sup>st</sup> time donor (n = 67)	Replacement donor (n = 43)	Voluntary donor (n = 19)		
MCV (fl)	81.7 ± 4.4	81.5 ± 4.2	82.5 ± 3.8	78.8 ± 4.9	0.02
MCH (pg)	27.4 ± 2.3	27.8 ± 2.0	27.9 ± 1.1	26.5 ± 2.9	0.1
RDW (%)	10.7 ± 0.8	10.5 ± 0.7	11.8 ± 1.0	10.9 ± 0.9	0.005
S. Ferritin (ug/L)	77.9 ± 72.5	92.7 ± 79.3	66.7 ± 61.5	23.7 ± 30.2	0.001
Platelets (10 <sup>9</sup> /L)	269.5 ± 62.1	271.4 ± 56.8	278.9 ± 65.6	257.1 ± 52.4	0.68
Pre donation Hb (gm/dl)	14.8 ± 0.8	14.8 ± 0.7	14.8 ± 0.8	14.6 ± 0.9	0.7
Hematocrit (%)	44 ± 2.7	43.2 ± 2.8	43.4 ± 2.5	43.3 ± 2.7	0.42

Using a cutoff point of <15ug/l for serum ferritin, iron deficiency anemia was recorded in 10.1% of non-regular blood donors while it was recorded in 73.9% of regular blood donors ( $p < 0.001$ ). See Table 3 and Table 4. Overall, iron deficiency anemia was recorded in 19.7% of the whole study sample of 152.

**Table 3: Iron Status among blood donor groups**

Iron Status	No Regular Donors			Regular donor (n = 23)	P value
	1 <sup>st</sup> time donor (n = 67)	Replacement donor (n = 43)	Voluntary donor (n = 19)		
Iron deficiency present n (%)*	6 (9%)	4 (9.3%)	3 (15.8%)	17 (73.9%)	< 0.001****
Reduced iron levels n (%)**	14 (20.9%)	8 (18.6%)	4 (21%)	1 (4.4%)	
No iron deficiency n (%)***	47 (70.1%)	31 (72.1%)	12 (63.2%)	5 (21.7%)	
TOTAL	67 (100%)	43 (100%)	19 (100%)	23 (100%)	

\*Iron Deficiency: (<15ug/l)

\*\*Reduced Iron Level: (15-30ug/l)

\*\*\*No iron Deficiency: (>30ug/l)

\*\*\*\*at 5% significance level

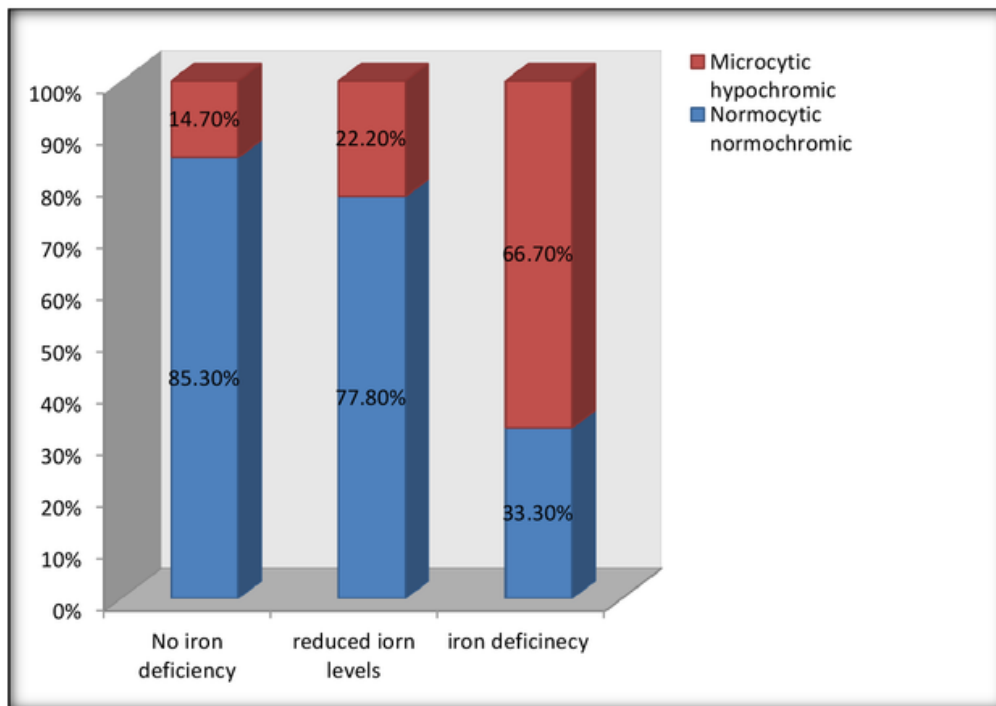
Table 4: Relationship between iron deficiency anemia and donor type

		Iron Deficiency Anemia		TOTAL	P value
		Yes	No		
Donor type	Non Regular Donor	13 (10.1%)	116 (89.9%)	129	< 0.001*
	Regular Donor	17 (73.9%)	06 (26.1%)	23	
	TOTAL	30 (19.7%)	122 (80.3%)	152	

\* Chi square test at 5% significance level

Pearson correlation test was applied at 5% level of significance to study the bivariate relationship between number of donations and its correlation with serum ferritin levels. It was observed that there is a weak but statistically significant inverse relationship existed between number of donations and serum ferritin levels. ( $r = -0.193$ ,  $p = 0.017$ ). Moreover, there was also weakly positive relationship between time since last donation (months) and serum ferritin levels (pearson  $r = 0.109$ ,  $p = 0.18$ )

Blood smear of all donors were examined for red cell morphology. In iron deficiency red blood cells appear microcytic and hypochromic. In this study, out of 62.5% blood donors with normal ferritin levels, 85.3% showed normal red cell morphology while 14.7% showed microcytic and hypochromic blood picture. Among iron deficient blood donors and those with reduced iron levels, 66.7% and 22.2% blood donors respectively showed microcytic hypochromic blood picture.



**Figure 1: Co-relation of peripheral smear findings to iron deficiency**

## Discussion:

Blood is donated as a “gift of life” therefore donor safety and protection should be of prime importance. <sup>26</sup> A number of studies have shown that frequent blood donation can cause sub-clinical iron deficiency <sup>33</sup>. To protect the donors from depletion of iron stores and development of iron deficiency, the donor selection criteria should have been revised but still current guidelines in most of the countries only require predonation haemoglobin measurement for donor selection <sup>6</sup>

<sup>20, 21</sup>

<sup>3</sup> The frequency of iron deficiency among blood donors differs, depending on many factors. The <sup>19</sup> REDS-II Donor Iron Status Evaluation (RISE) study revealed that 47% of frequent male donors <sup>32</sup> were iron deficient <sup>4</sup>. The prevalence of iron deficiency was higher in RISE study, compared to this study as 19.73% of our study population were iron deficient. This can be due to number of reasons i.e. RISE study used a number of iron parameters with different reference ranges, also hemoglobin level set for donation was low 12.5g/dl compared to current study.

Descriptive analysis was done, first among regular and non-regular blood donors and then between the subgroups-of non-regular donors. Taking out the mean value of different biochemical and hematological parameters used in this study, it was recorded that the mean values were lower for all the variables in blood samples of the regular donors as opposed to non-regular donors. Same results were observed in blood donors of Saudi<sup>10</sup> and Nigeria<sup>3</sup>.

The comparison of serum ferritin values among sub-groups of non-regular donors showed that 1<sup>st</sup> time donors and replacement donors are safer groups for blood donation compared to voluntary donors. This may be because voluntary donors had overall more donations done as compared to the other two groups. Also their time since last donation was also less as compared to the other



groups.<sup>4, 10</sup> Our study shows that <sup>43</sup>depletion of iron stores is related to <sup>7</sup>increasing number of blood donations in last 12 months. This is comparable to a study done in Hong Kong<sup>21</sup>.

In this study 19.7% of the male blood donors meeting the selection criteria with normal haemoglobin level had sub-clinical iron deficiency. This shows that haemoglobin level does not directly associate with iron status<sup>4, 20, 21</sup>

Current study also shows that serum ferritin levels are much lower in regular donors as compared to non-regular donors. This finding also correlates with the results reported in a number of other studies<sup>8, 20</sup>. Similarly a local study done in Pakistan demonstrated that, blood <sup>31</sup>donors who have <sup>30</sup>donated blood four times or more in last two years, are likely to develop iron deficiency and iron deficiency anemia as compared to those who donated less frequently<sup>22</sup>.

This study demonstrated that the total number of blood donations also affects the iron status as 73.9% of regular blood donors while 15.8% voluntary donors and 9% each of the 1<sup>st</sup> time and replacement donor were found to be iron deficient. A similar study done in Iran favours our findings<sup>23</sup>.

Further large scale studies with multiple parameter analysis including haemoglobin, ferritin, iron binding capacity, hepcidin, erythropoietin, soluble transferrin before donation and after donation are recommended for future research to determine the exact time needed for these parameters to return to normal.

## Conclusion

Hemoglobin and red cell indices are not sufficient to determine the eligibility for donation.

Regular <sup>25</sup> donors are at risk of developing iron deficiency. Measurement <sup>of</sup> serum ferritin should be included in donor selection criteria to prevent sub-clinical iron deficiency and to protect them from anemia due to frequent blood donations. Government should either perform these tests free of cost to keep flow of donors. In the absence of ferritin assesment due to cost constraints <sup>7</sup> prolongation of donation interval and advice on iron repletion is a necessary for donor welfare.

## Conflict of intrest

Authors declare no conflict of intrest

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