Altered Red Cell Indices in Repeat Blood Donors: Experience of a North Indian Blood Bank

Ashish Jain¹ · Nilotpall Chowdhury² · Sanchit Jain³ · Nikita Uttam¹ · Sushant Kumar Meinia¹

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Abstract Subclinical Iron deficiency appearing in blood donors after blood donation is a recognized problem. Donors at an increased risk of iron deficiency need to be identified. Blood donors meeting national selection criteria were included in the study. Complete blood counts were done using Sysmex XP-100 three part hematology analyzer. Differences in RBC indices among donor groups defined by previous donations were then analyzed statistically. Six hundred and seventy three males and ninety six females were studied. In males, Kruskal–Wallis test showed significant differences between groups defined by number of donations for MCH and MCV (P value < 0.001), but not for MCHC (P value $= 0.09$) and RDW (P value $= 0.6$). Post-hoc tests showed statistically significant difference between donors having six or more donations compared to donors having no previous donations for both MCH as well as MCV. No significant difference was found between donor subgroups in females for any of the indices; however, no female donated blood more than three times in the study. There is increased risk for iron deficiency in donors having six or more previous donations, and evidence for starting an iron screening and supplementation programme for these donors.

Keywords Anemia · Iron-deficiency · Blood donors · Blood cell count · Erythrocyte indices

Introduction

Subclinical Iron deficiency appearing in blood donors after phlebotomy is a recognized problem. Repeat donors are likely to be at a greater risk of iron deficiency. It is important to identify which donors are in need of iron supplementation. Iron deficiency may be identified in a subclinical stage by measurement of RBC indices such as Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC) or Red Cell Distribution Width (RDW) [1–3].

The present study explored the relationship between the RBC indices (serving as a marker of iron deficiency) with the number of donations given by a blood donor. It was hoped that this would lead to the identification of a subgroup of donors who would be at an increased risk of iron deficiency and thus would benefit most from iron supplementation.

Materials and Methods

This was a cross-sectional study done on blood donors in our blood bank between September 2016 and April 2017. Ethical clearance to publish this study was taken from the institute ethics committee.

All blood donors who were found suitable for blood donation after following the national regulatory guidelines were included in the study [4]. All donors were between 18 and 60 years of age, had a hemoglobin greater than 12.5 g/dL (on assessment of capillary hemoglobin by HemoCue devices), were clinically healthy with no clinical evidence of acute or chronic disease, and had a gap of at least 3 months compared to the previous donation. History of

✉ Ashish Jain
ashish.jain.modi@gmail.com

¹ Department of Transfusion Medicine and Blood Bank, All India Institute of Medical Sciences, Rishikesh, Uttarakhand 249201, India

² Department of Pathology and Laboratory Medicine and Blood Bank Incharge, All India Institute of Medical Sciences, Rishikesh, India

³ All India Institute of Medical Sciences, Rishikesh, India

Table 1 The distribution of the RBC indices among the groups defined by number of donations in males

	Previous donations	Number of donors	Mean (SD)	Median [minimum, maximum]
MCH (in pg) K–W <i>P</i> value < 0.001	0	358	30.1 (2.7)	29.8 [20.1,41.8]
	1	174	30.4 (2.7)	30.4 [20.5, 40.5]
	2–3	68	29.7 (3.2)	29.9 [22.1, 40.0]
	4–5	38	31.0 (2.9)	30.4 [26.0,39.8]
	6 or greater*	35	28.3 (2.5)	27.8 [21.9, 36.2]
MCHC (in g/dL) K–W <i>P</i> value 0.09	0	358	34.5 (1.2)	34.5 [29.3, 38.3]
	1	174	34.7 (1.2)	34.7 [30.7, 37.9]
	2–3	68	34.2 (1.6)	34.5 [29.6, 38]
	4–5	38	34.7 (1.1)	34.6 [31.9, 36.9]
	6 or greater	35	34.1 (1.1)	34.1 [30.8, 36.0]
MCV(in fL) K–W <i>P</i> value < 0.001	0	358	87.2 (6.3)	86.7 [64.9, 114.2]
	1	174	87.8 (6.2)	87.3 [66.7, 113.1]
	2–3	68	86.7 (7.3)	86.0 [69.9, 110.4]
	4–5	38	89.2 (6.6)	89.0 [78.4, 108.3]
	6 or greater*	35	82.9 (6.0)	83.1 [71.1, 104.2]
RDW (in %) K–W <i>P</i> value 0.6	0	358	14.0 (1.2)	13.8 [11.9, 19.1]
	1	174	14.0 (1.2)	13.7 [11.9, 18.8]
	2–3	68	14.3 (1.6)	14.1 [12.4, 23.0]
	4–5	38	14.1 (1.2)	13.9 [12.0, 17.0]
	6 or greater	35	14.3 (1.7)	13.9 [12.1, 22.2]
HGB (in g/dL) K–W <i>P</i> value 0.014	0	358	15.4 (1.2)	15.5 [12.3, 21.2]
	1	174	15.6 (1.2)	15.7 [12.8,20.6]
	2–3	68	15.2 (1.2)	15.3 [12.0, 18.5]
	4–5	38	15.5 (1.3)	15.6 [12.8, 18]
	6 or greater*	35	14.9 (0.9)	14.9 [12.3, 16.9]
RBC (in 10 ⁶ /cu mm) K–W <i>P</i> value 0.4	0	358	5.2 (0.5)	5.2 [3.3, 6.8]
	1	174	5.1 (0.6)	5.2 [3.4, 6.7]
	2–3	68	5.2 (0.6)	5.2 [3.1, 6.7]
	4–5	38	5.0 (0.5)	5.0 [3.3, 6.2]
	6 or greater	35	5.3 (0.4)	5.3 [4.3, 6.3]

K–W *P* val = the *P* value of the Kruskal–Wallis test for differences among the various groups, the asterisk denotes the groups which showed significant difference against the control group of no previous donations at a Holm–Bonferroni corrected value of 0.05

number of previous blood donations by the donor was taken. Three ml of discarded blood from the diversion pouch in EDTA vials were taken and analyzed by Sysmex XP-100 three part hematology analyzer. The readings for Hemoglobin (HGB), RBC Count (RBC), MCH, MCHC, MCV and RDW were taken for this study.

These parameters were then analyzed statistically, after eliminating large outliers, comparing blood donors grouped according to the number of donations they had previously given. Five groups of donors were compared, the first group consisting of first time donors, the second group of those who had donated just once previously, the third group of those who had donated two to three times previously, the fourth group of those who had donated four to five times

previously, the fifth group of those who had donated six or more times previously.

Kruskal–Wallis test was used to assess whether the RBC indices differed significantly between these groups among male and female donors. A significant Kruskal–Wallis test (at a *P* value of 0.05) was followed by post hoc Dunn test against the control population of first time donors with Holm adjustment for multiple comparisons.

Finally, microcytosis below 80 fL (the lower reference limit validated in our laboratory) along with Mentzer index [5] of greater than 13 was taken as a proxy marker of iron deficiency, and in order to differentiate from thalassemia trait. The equality of proportion of estimated iron deficiency among the different groups was tested by the Fisher exact test. The odds ratio of the estimated iron deficiency in

Table 2 The distribution of the RBC indices among the groups defined by number of donations in females

	Previous donations	Number of donors	Mean (SD)	Median [minimum, maximum]
MCH (in pg) K–W <i>P</i> value 0.1	0	72	29.6 (2.4)	29.7 [23.4, 36]
	1	19	29.6 (1.8)	30.3 [25.7, 33.4]
	2–3	5	27.8 (1.7)	28.6 [24.9, 29.1]
MCHC (in g/dL) K–W <i>P</i> value 0.08	0	72	34.2 (1.1)	34.4 [31.3, 36.6]
	1	19	34.4 (0.9)	34.5 [32, 35.6]
	2–3	5	33.1 (0.9)	33.4 [31.9, 34]
MCV (in fL) K–W <i>P</i> value 0.5	0	72	86.6 (5.5)	86.2 [71.3, 103.7]
	1	19	86.2 (4.4)	86.7 [78, 99.8]
	2–3	5	84.0 (3.7)	85 [78.2, 88.1]
RD (in %) K–W <i>P</i> value 0.7	0	72	13.8 (1.2)	13.6 [11.3, 17.7]
	1	19	13.9 (0.9)	13.9 [12.8, 15.5]
	2–3	5	14.0 (0.6)	14.2 [13, 14.4]
HGB (in g/dL) K–W <i>P</i> value 0.6	0	72	13.6 (0.9)	13.4 [12.1, 17.7]
	1	19	13.4 (0.7)	13.3 [12.5, 15]
	2–3	5	13.4 (0.3)	13.5 [12.9, 13.8]
RBC (in 10 ⁶ /cu mm) K–W <i>P</i> value 0.1	0	72	4.6 (0.4)	4.6 [3.6, 5.7]
	1	19	4.5 (0.3)	4.5 [4.1, 5.1]
	2–3	5	4.8 (0.2)	4.7 [4.6, 5.2]

K–W *P* value = the *P* value of the Kruskal–Wallis test for differences among the various groups

the different donor groups against the control group having no previous donation was estimated along with mid-*P* exact 95% Confidence Intervals and mid-*p* *P* values.

MCV between 72 and 80 fL was taken as a second proxy marker of iron deficiency, since a cut-off of about 72 fL has been demonstrated to be useful in differentiating iron deficiency from thalassemia trait [6, 7], and has text book support in this regard [8].

All the tests were done using R statistical environment [9], along with the PMCMR [10] and epitools [11] packages.

Results

Six hundred and seventy three males and ninety six females were examined in the present study.

The Kruskal–Wallis test showed significant differences between the groups defined by number of donations for MCH, MCV, and HGB but not for MCHC, RDW and RBC (Table 1). Post Hoc Tests by Dunn's method showed statistically significant difference between the donors having 6 or more donations compared to donors having no previous donations for MCH, MCV and HGB.

We did not get any female donor having more than 3 previous donations in the time period of the study. There was no significant difference found between subgroups for any of the parameters by the Kruskal–Wallis test (Table 2).

The groups having six or more previous blood donations had large statistically significant odds ratios for iron deficiency using Mentzer index of greater than 13 combined with a MCV of less than 80 fL as a proxy marker for iron deficient state (Table 3). The odds ratios for the other groups were neither large, nor statistically significant. Similar results were obtained when the second proxy marker for iron deficiency was tested among the different groups (overall *P* value by Fisher's exact test = 0.007 in males and 0.4 in females, Odds ratio for group having 6 or more previous donations versus no donation = 4.39 with a mid-*p* *P* value of 0.002).

Discussion

The primary finding of this study is that donors having donated blood more than five times previously have significantly lower MCH and MCV compared to those having no previous donation. This suggests that these donors are more likely to have of subclinical iron deficiency and are likely candidates for iron supplementation.

The groups having more than five previous blood donations, but not the other groups, had large statistically significant odds ratios for estimated iron deficiency by two proxy markers (combined microcytosis with Mentzer's index greater than 13, and MCV between 72 and 80 fL). This supports the contention that donors having more than

Table 3 The odds ratio of finding iron deficiency (as estimated by a proxy of Mentzer Index less than 13 and microcytosis less than 80 fL) in the various groups defined by previous blood donation, with the

mid-p 95% confidence intervals (CI) of the odds ratio, compared to the control group having no previous donation, for all donors

Previous donations	Males			Females		
	IDA	Odds ratio [lower CI, upper CI]	Exact mid-p value	IDA	Odds ratio [lower CI, upper CI]	Exact mid-p value
0	21/358	–	–	8/72	–	–
1	6/174	0.57 [0.21, 1.40]	0.2	1/19	0.45 [0.02, 3.07]	0.5
2–3	6/68	1.55 [0.55, 3.88]	0.4	1/5	1.98 [0.07, 18.19]	0.6
4–5	1/38	0.43 [0.02, 2.46]	0.5			
6 or greater	7/35	3.99 [1.46, 10.03]	0.009			
Overall <i>P</i> value	0.007			0.4		

IDA = Proportion of donors predicted to be have Iron deficiency by the proxy. The overall *P* value for difference in proportions is estimated by Fisher exact test

5 donations need priority over others in screening for iron deficiency and iron supplementation.

RDW has been suggested as a better marker for early diagnosis of iron deficiency compared to MCV or MCH [2, 12, 13]. However, the present study did not find significant differences in RDW among the various donor groups. We are unable to explain this non-finding; a possible reason may be that RDW is increased only in a more rapidly progressive iron deficiency, but not in a chronic subclinical deficiency state.

MCHC is a relatively insensitive marker of iron status compared to the MCH or MCV [13]; significant difference between the donor groups for MCHC was not found, although a trend towards significance could be observed.

We found no significant differences among the donation defined groups in the females, probably because there were no females having more than previous three blood donations. Nine out of 72 (12.5%) of female blood donors having no previous blood donation had MCV less than 80 fL and possibly may already have subclinical iron deficiency. Whether these donors develop overt iron deficiency after the first donation needs to be examined in a follow up study.

Chemical estimation of ferritin and other recent markers like Reticulocyte hemoglobin concentration, are more accurate markers of iron deficiency [14, 15], and may be used to confirm findings of the present study. However, we believe that the present study gives sufficient evidence in favor of starting a directed, focused donor screening program using serum ferritin in donors having more than five previous donations.

To conclude, we found evidence of increased risk for iron deficient state in donors having six or more previous donations. However, follow up of iron status of first time

donors, especially females with chemical confirmation, may further supplement the present study.

Compliance with Ethical Standards

Ethical Standard Ethical clearance for this study was taken from the institute ethics committee.

Conflict of interest None.

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