Research Article

Red blood cell indices and red blood cell morphology in the haematological evaluation of patients: Study from a tribal based hospital in rural India

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ABSTRACT

Background: Anaemia causes impaired oxygen delivery to tissues. The first investigation done in anaemia is blood for haemoglobin%(Hb%). It can be reported within minutes using automated analyser. Peripheral blood smear (PBS) examination for assessment of red cell morphology is advised to evaluate the effect of anaemia on patient's health. Now-a-days, automated analysers are supplied in remote hospital set-up also. But analyser results must be justified with blood smear examination findings. **Objectives:** General: To find out the spectrum of Hb concentration among indoor patients; Specific: To find out whether analyser derived red cell indices correspond with red cell morphology on PBS. **Materials and Methods:** Observational, cross-sectional, descriptive study conducted in the pathology department of a tribal based hospital. After institutional ethics committee permission the study was conducted for 1 year duration among indoor patients selecting the first complete haemogram report after admission. Hb values were categorised into 4 sub-groups (≥12 g/dl, 10–<12 g/dl, 7–<10 g/dl, <7 g/dl). Red cell indices were derived from analyser. Blood smear stained by Leishman-Giemsa was examined under light microscope. Data was analysed at the end of the study using Microsoft Excel software 2010. **Results:** Among 478 total cases 39.53% were male, 60.46% female (Male:Female ratio 1:1.53). 50% of total population had Hb% ≥12 g/dl which constituted 69.84% of the male and 37.02% of the female cases. Females were more in number to have abnormal red cell morphology (23.53%) compared to male (19.05%). Red cell index abnormality was almost equal in male (58.20%) and female (59.52%). More male patients had red cell morphology abnormality (54.38%) than female (34.62%) at Hb level <12g/dl. **Conclusion:** Red cell morphology must accompany all cases of haemogram reporting.

KEY WORDS: Anaemia; Haemoglobin; Peripheral Blood Smear; Red Cell Indices; Red Cell Morphology

INTRODUCTION

Anaemia is not a disease in itself, but a manifestation of many underlying pathologies. Here, the red blood cell (RBC) mass is insufficient to deliver oxygen to the tissues. That may be due to reduced RBC mass and/or reduced haemoglobin (Hb) content of RBC. Management of anaemia begins with laboratory assessment of complete blood count (CBC) that includes Hb%, RBC count, RBC indices, total leucocyte count, differential leucocyte count, platelet count, reticulocyte count. RBC indices include mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and red cell distribution width (RDW). RBC morphology is assessed from peripheral blood smear (PBS) under light microscope. The reference ranges of Hb% for men and women are 15 ± 2 g/dl and 13.5 ± 1.5 g/dl respectively.[1] Anaemia is defined when Hb% is <13 g/dl for men and <12 g/dl for women.[2] The RBC transfusion threshold for most patients has been found to remain between Hb% 7 g/dl and 10 g/dl.[3] Hence, the clinicians need to identify the tolerable Hb% level in each patient so as to avoid hazards of transfusion.

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Previously, all the components of CBC were calculated manually. With the introduction of automated haematology analyser the reporting of CBC has become faster. It minimized manual error and saved many working hours. RDW is the new RBC index that is completely machine derived. It correlates with RBC size variation and hence gives indication regarding the degree of anisocytosis. Several studies have shown that long term sample storage, presence of RBC agglutinin, RBC fragmentation, hyperglycaemia, hyperlipidaemia, high lymphocyte count can affect analyser estimates.[4-6] Giant platelets are mistaken by analyser as micro-erythrocyte giving rise to raised RDW. Presence of malaria parasite within the RBC increases RBC size and may give false impression of macrocytosis and hypochromia in the analyser results. These can lead to altered MCV, MCH values. Hence examination of PBS is important. It sometimes guides the clinician regarding the underlying aetiology or urgency of transfusion requirements. Now-a-days, automated analysers are supplied even in remote hospital set-up so as to have quick reporting and better patient management. But analyser results are not to be followed blindly. They must be justified with blood smear examination findings.

We undertook one observational, cross-sectional, descriptive study to find out whether reporting of RBC indices from automated haematology analyser and the RBC morphology on PBS are contemporary to each other or not. Objectives of the study were as follows: General: To find out spectrum of Hb concentration among indoor patients; Specific: To find out whether analyser derived RBC indices correspond with RBC morphology on PBS.

MATERIALS AND METHODS

Following institutional ethical committee permission the study was conducted in the department of pathology for 1 year duration. All the indoor cases advised for CBC were included. Only the first report of CBC after admission was selected. The patients receiving blood transfusion in the last 7 days, known cases of malignancy, haemolysed sample, clotted sample, sample insufficient to run in analyser were excluded from the study. Hb% values were categorized into 4 subgroups (≥12 g/dl, 10–<12 g/dl, 7–<10 g/dl, <7 g/dl) taking into consideration the transfusion trigger at Hb% of 7–10g/dl.[3] RBC indices were estimated from automated analyser. PBSs were prepared, stained with Leishman-Giemsa stain and examined under light microscope. Normal RBC size is comparable to the nucleus of a small mature lymphocyte in PBS.[7,8] RBCs with size smaller and larger than this are described as microcytes and macrocytes respectively. Chromicity of RBC is assessed from the central pallor. In the present study normocytic normochromic smear was categorized as normal RBC morphology, while any deviation as abnormal morphology.

Statistics

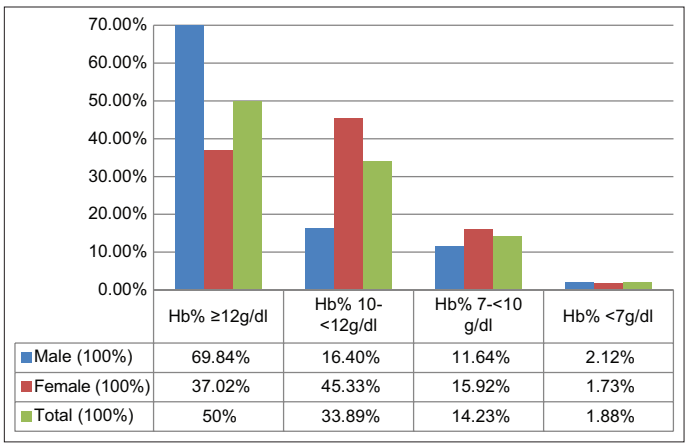
Data was analysed at the end of the study using Microsoft Excel software 2010.

RESULTS

In the study we had total 478 patients, among them 189 (39.53%) were male and 289 (60.46%) were female. Male/female ratio was 1:1.53 [Table 1]. Among total female patients 37.02% had Hb% ≥12 g/dl, 45.33% from 10–<12 g/dl, 15.92% from 7–<10 g/dl and 1.73% had Hb% <7 g/dl. Among the male patients 69.84% had Hb% ≥12 g/dl, 16.40% from 10–<12 g/dl, 11.64% from 7–<10 g/dl and 2.12% had <7g/dl [Figure 1]. We found 50% of total population had Hb% ≥12 g/dl. At Hb% <12 g/dl we had 30.16% of total male and 62.98% of total female patients (M:F = 1:2.09) but at Hb% ≥12 g/dl 69.84% of total male and 37.02% of total female patients (M:F = 1.89:1). If we classify the population by Hb% <12 and ≥12 g/dl, we had male patients at 1:2.32 ratio and female patients at 1.70:1 ratio. So, female patients had more chance to have Hb% <12 g/dl.

Among the male patients 80.95% had normal RBC morphology and 19.05% abnormal morphology that included anisocytosis, microcytosis, macrocytosis, poikilocytosis, hypochromia in varying combination. Among female patients, these were 76.47% and 23.53% respectively. So, abnormal RBC morphology among male and female patients were 19.05% and 23.53% respectively (M:F = 1:1.24). Overall female patients were more susceptible to develop abnormal morphology than male patients.

While studying the 4 categories of cases closely we found that among male patients at Hb% ≥12 g/dl 96.21% had normal and 3.79% abnormal RBC morphology; at Hb% 10–<12 g/dl 58.06% had normal and 41.94% abnormal RBC morphology; at Hb% 7–<10 g/dl 36.36% had normal and 63.64% abnormal morphology; at Hb% <7 g/dl all the



	Hb% ≥12g/dl	Hb% 10-<12g/dl	Hb% 7-<10 g/dl	Hb% <7g/dl
Male (100%)	69.84%	16.40%	11.64%	2.12%
Female (100%)	37.02%	45.33%	15.92%	1.73%
Total (100%)	50%	33.89%	14.23%	1.88%

Figure 1: 'Bar diagram showing distribution of total population according to gender and hemoglobin (g/dl) level. X-axis: haemoglobin %, Y-axis: percentage of cases

Table 1: Distribution of total population according to Age (Years), Sex, Hb (g/dl) and RBC morphology (*n*=478)

Age (Years)	Hb (g/dl)																Total (%)
	Male								Female								
	≥12		10–<12		7–<10		<7		≥12		10–<12		7–<10		<7		
	N	A	N	A	N	A	N	A	N	A	N	A	N	A	N	A	
<20	2	1	0	0	0	0	0	0	1	0	2	0	0	1	0	0	7 (1.46)
20–<30	20	1	2	0	0	2	0	0	26	0	19	6	1	4	0	0	81 (16.95)
30–<40	28	0	2	3	0	2	0	0	23	0	15	8	2	6	1	0	90 (18.83)
40–<50	19	0	1	1	3	0	0	0	19	3	23	10	5	6	0	2	92 (19.25)
50–<60	22	2	1	1	0	1	0	1	9	1	16	3	4	5	0	1	67 (14.02)
60–<70	19	1	5	6	2	3	0	2	17	1	19	2	4	4	0	1	86 (17.99)
≥70	17	0	7	2	3	6	0	1	7	0	6	2	2	2	0	0	55 (11.50)
Total	127	5	18	13	8	14	0	4	102	5	100	31	18	28	1	4	478 (100)
	132		31		22		4		107		131		46		5		
	189 (39.53%)								289 (60.46%)								

N: Normal RBC morphology, A: Abnormal RBC morphology, Hb: Hemoglobin, RBC: Red blood cell

cases (100%) had abnormal RBC morphology. Among the female patients we found that cases at Hb% ≥12 g/dl 95.33% had normal and 4.67% abnormal RBC morphology; at Hb% 10–<12 g/dl 76.34% had normal and 23.66% abnormal RBC morphology; at Hb% 7–<10 g/dl 39.13% had normal and 60.87% abnormal RBC morphology and at Hb% <7 g/dl 20% of the cases had normal and 80% abnormal RBC morphology.

Now while studying the RBC index we found that among all female patients 40.48% had normal and 59.52% abnormal RBC index. Among the female cases with Hb% ≥12 g/dl 65.42% had normal and 34.58% abnormal RBC index; Hb% 10–<12 g/dl 29.77% had normal and 70.23% abnormal RBC index; Hb% 7–<10 g/dl 17.39% had normal and 82.61% abnormal RBC index. All the cases with Hb% <7 g/dl had abnormal RBC index (100%). The male population also showed similar kind of trends. Among all male patients 41.80% had normal and 58.20% abnormal RBC index. For the group with Hb% ≥12 g/dl 53.79% had normal and 46.21% abnormal RBC index; Hb% 10–<12 g/dl 19.35% had normal and 80.65% abnormal RBC index; Hb% 7–<10 g/dl 9.09% had normal and 90.91% abnormal RBC index. All the cases (100%) with Hb% value <7 g/dl had abnormal index [Table 2]. Overall, the RBC index abnormality was almost equal in male (58.20%) and female (59.52%) groups.

59.52% of total female patients had abnormal RBC index while only 23.53% had abnormal RBC morphology. In the non-anaemic female group (Hb% ≥12 g/dl) 34.58% had abnormal index, but only 4.67% had abnormal morphology. At Hb% 10–<12 g/dl, 70.23% had abnormal RBC indices, but on PBS examination the abnormality was found to be 23.66%. Hb% 7–<10 g/dl group showed 82.61% cases with abnormal indices and 60.87% abnormal morphology. We had only 5 cases with Hb% value <7 g/dl. All of them had abnormal RBC indices, but PBS showed 20% cases with normal morphology. So there was always a disparity between abnormal RBC index and morphology. This gap narrowed down with fall of Hb%.

Our study described 58.20% of all male patients had abnormal RBC indices, but PBS examination showed only 19.05% had abnormal morphology. 46.21% of the cases with Hb% ≥12 g/dl had abnormal RBC indices, while only 3.79% had abnormal RBC morphology. In the group of Hb% 10–<12 g/dl 80.65% had abnormal RBC indices, but on PBS examination only 41.94% showed abnormality. At Hb% 7–<10 g/dl 90.91% cases had abnormality in indices, which came down to 63.64% on morphology. We had only 4 cases with Hb% value <7 g/dl. All of them had abnormal RBC indices as well as abnormal morphology. The disparity between index and morphology abnormality reduces with fall of Hb%.

Our study showed with fall of Hb% RBC morphology abnormality increases in both male and female. On close evaluation of the results an interesting finding came out. At Hb% <12 g/dl more female patients had normal RBC morphology compared to male patients. In other words, RBC morphology abnormality was more in male (54.38%) than female (34.61%) at Hb% <12 g/dl. At Hb% ≥12 g/dl morphology abnormality was almost equal in both male (3.79%) and female cases (4.67%) [Table 3]. Every group analysis showed percentage of cases with abnormal RBC indices reduces further on PBS examination [Table 4].

DISCUSSION

Our study was dominated by female group of population (60.46%) majority of whom (62.98%) had Hb% <12 g/dl, in contrast to male patients where most of the cases (69.84%) had Hb% ≥12 g/dl. Male/female ratio of our study was 1:1.53. Overall, on analysing RBC morphology the abnormality was found to be more in female patients (23.53%) compared to

Table 2: Distribution of total population according to age (years), Sex, Hb (g/dl) and RBC index (*n*=478)

Age (Years)	Hb (g/dl)																Total (%)
	Male								Female								
	≥12		10–<12		7–<10		<7		≥12		10–<12		7–<10		<7		
	N_1	A_1	N_1	A_1	N_1	A_1	N_1	A_1	N_1	A_1	N_1	A_1	N_1	A_1	N_1	A_1	
<20	0	3	0	0	0	0	0	0	0	1	0	2	0	1	0	0	7 (1.46)
20–<30	11	10	0	2	1	1	0	0	17	9	10	15	0	5	0	0	81 (16.95)
30–<40	14	14	0	5	0	2	0	0	14	9	5	18	0	8	0	1	90 (18.83)
40–<50	8	11	0	2	0	3	0	0	13	9	8	25	2	9	0	2	92 (19.25)
50–<60	12	12	1	1	0	1	0	1	8	2	6	13	4	5	0	1	67 (14.02)
60–<70	13	7	3	8	0	5	0	2	13	5	6	15	1	7	0	1	86 (17.99)
≥70	13	4	2	7	1	8	0	1	5	2	4	4	1	3	0	0	55 (11.50)
Total	71	61	6	25	2	20	0	4	70	37	39	92	8	38	0	5	478 (100)
	132		31		22		4		107		131		46		5		
	189 (39.53%)								289 (60.46%)								

N_1: Normal RBC index, A_1: Abnormal RBC index, Hb: Hemoglobin, RBC: Red blood cell

Table 3: Comparison of male and female population according to Hb% and RBC morphology changes

Hb (g/dl)	Patients	RBC morphology		Total (%)
		Normal (%)	Abnormal (%)	
≥12	Male	127 (96.21)	5 (3.79)	132 (100)
	Female	102 (95.33)	5 (4.67)	107 (100)
<12	Male	26 (45.61)	31 (54.38)	57 (100)
	Female	119 (65.38)	63 (34.62)	182 (100)

Hb: Hemoglobin, RBC: Red blood cell

Table 4: Comparison of male and female population according to Hb%, RBC index and RBC morphology

Hb (g/dl)	Feature	Normal (%)	Abnormal (%)	Total (%)
≥12				
Male	RBC index	71 (53.79)	61 (46.21)	132 (100)
	RBC morphology	127 (96.21)	5 (3.79)	132 (100)
Female	RBC index	70 (65.42)	37 (34.58)	107 (100)
	RBC morphology	102 (95.33)	5 (4.67)	107 (100)
10–<12				
Male	RBC index	6 (19.35)	25 (80.65)	31 (100)
	RBC morphology	18 (58.06)	13 (41.94)	31 (100)
Female	RBC index	39 (29.77)	92 (70.23)	131 (100)
	RBC morphology	100 (76.34)	31 (23.66)	131 (100)
7–<10				
Male	RBC index	2 (9.09)	20 (90.91)	22 (100)
	RBC morphology	8 (36.36)	14 (63.64)	22 (100)
Female	RBC index	8 (17.39)	38 (82.61)	46 (100)
	RBC morphology	18 (39.13)	28 (60.87)	46 (100)
<7				
Male	RBC index	0 (0)	4 (100)	4 (100)
	RBC morphology	0 (0)	4 (100)	4 (100)
Female	RBC index	0 (0)	5 (100)	5 (100)
	RBC morphology	1 (20)	4 (80)	5 (100)

Hb: Hemoglobin, RBC: Red blood cell

male (19.05%). At Hb% ≥12 g/dl normal RBC morphology was almost equal in male (96.21%) and female cases (95.33%). But, at Hb% <12 g/dl morphology abnormality was more common in male (54.38%) than female (34.61%) patients. Fall of Hb% affects male RBC morphology earlier than female RBCs. It could also be said that morphological abnormality appears later in female RBCs compared to male RBCs. In female patients some compensatory mechanism might be active before developing morphological abnormality. We can hypothesize that female RBCs become more adjusted with the cyclical blood loss.

Among all cases, abnormal RBC index was equal in male (58.20%) and female (59.52%). The index abnormality grew higher with fall of Hb% in both the cases. At Hb% ≥12 g/dl the index abnormality was very high in male (46.21%) and female (34.58%) groups, but morphology abnormality was low and almost equal in both the cases (male = 3.79% and female = 4.67%). As we had categorised total population into 4 groups based on Hb%, some of the male patients in the Hb% ≥12 g/dl group were having less than normal Hb% value for male (12–13 g/dl). This might be the reason of very high abnormal RBC index compared to morphology in this group. There was always a disparity between RBC index and abnormal morphology which was wider at higher Hb% value and narrowed down with fall of Hb%. So, alteration of Hb% affects RBC index earlier, morphology abnormality appears later.

Saichanma *et al*.[9] in Thailand had studied teenage group of cases and evaluated 1362 cases where they found 19.1% male, 80.9% female. Abnormal RBC morphology was found in 25.99% of total cases that included 2.64% male and 23.35% female. Our findings were a bit closer to their study, though here age ranged from <20 to >70 years with majority in 40–50 years followed by 30–40 years group. 23.53% of total female cases had abnormal RBC morphology in our study. Ashok and Varadaranjan.[10] also found majority

of the patients in the 41–50 years age group. Sandhya and Rashmi.[11] had similar kind of findings with majority between 41 and 50 years followed by 31–40 years. Our study had 39.53% male and 60.46% female patients among total population with male:female ratio (M:F) 1:1.53. Nanwani and Khatri.[12] had M:F ratio 1:1.75. Hafiz *et al*.[13] had the ratio 1:1.22. Kumari and Mohan.[14] studied 260 cases and found 38% male and 62% female with M:F ratio of 1:1.6. Barve *et al*.[15] also found female patients more than male patients (F>M). Sandhya and Rashmi.[11] had 51.4% female and 48.6% male. Singla *et al*.[16] studied 500 patients and found 53.6% female and 46.4% male (F>M). There were 77% female and 23% male patients (F>M) in the study by Ashok and Varadaranjan.[10] Pudasaini *et al*.[17] found 68.2% female and 31.8% male among 274 cases. Choudhary *et al*.[18] also had F>M cases.

In the review article Constantino[8] had emphasized the importance of morphological characterization in PBS despite tremendous upgradation of automated hematology analyser. Nanwani and Khatri.[12] in their study concluded that PBS findings and analyser derived findings (histogram) are supplementary to each other. Both in combination help in correct diagnosis of majority of anaemia. Kumar *et al*.[19] discussed the role of PBS examination in the investigation of haematological disorder and concluded that PBS and analyser together can help in better patient diagnosis. Researchers have emphasized several times that PBS examination is a critical step in evaluation of patients.[11,20-22] Ashok and Varadaranjan.[10] also concluded that PBS examination still an important diagnostic tool even in the era of automation. Hafiz *et al*.[13] highlighted the importance of PBS examination even in the presence of analyser reporting. Kumari and Mohan.[14] also emphasized the importance of microscopic examination of PBS.

In short, all these studies have pointed out that female patients outnumber the male patients and PBS examination is important whatever be the modernisation of the analyser. Present study has also highlighted similar findings. We have pointed out that with fall of Hb% the disparity between abnormal RBC index and morphology reduces. In addition we have found that morphological abnormality is more in male than female RBCs at Hb% level <12 g/dl.

Strength and Limitations of Present Study

The study was conducted among hospital admitted patients. Hence, the cases were mixed up from several departments. Also we were able to collect large number of cases in a short time span despite having several exclusion criteria. But the study was severely limited financially. We couldn't have studied a large number of parameters which might have shown some additional implications. If the individual parameters could be analysed separately and correlated with the PBS findings, then a general approach to complete haemogram might have been established. We had broadly classified RBC morphology as normal and abnormal. Specific categorisation and comparison with individual indices need to be done to point out any trivial difference. The scope of study needs to be expanded to a large scale of population along with adequate financial support, so that a general inference can be demonstrated. Hence the study needs to be undertaken further on a large population size on general community.

So, if we routinely examine PBS of all cases of haematology samples we can classify patient's condition much better. Further clinicians can take a more conservative approach in management of anaemia cases and defer transfusion as far as possible. Leaving aside male and female categorisation, if we consider whole of the study population it is evident that fall of Hb% affects RBC index earlier. Morphology abnormality appears much later. This might be because of the fact that indices are derived from more than one factor. Examination of morphology on peripheral smear is a bit straight forward. So, if we combine RBC morphology on PBS with the RBC indices in every case, we can have a better picture of patient's haemogram status.

CONCLUSION

From the present study we conclude that PBS examination should be made mandatory in all cases of haemogram analysis. It's not the machine, but man behind the machine is important.

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