BONE MARROW IRON STORE IN PERLS' STAINED ASPIRATES AND THE RED CELL INDICES

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ABSTRACT

BACKGROUND

The National Family Health Survey 3 (NFHS-3) states the prevalence of anaemia in India to be 55.3% in women and 24.2% in men. The evaluation of Bone Marrow (BM) iron is a sensitive and reliable means for the diagnosis of iron deficiency anaemia. BM iron identified by Perls' Prussian blue reaction is regarded as the gold standard for evaluating BM iron stores. Surrogate serum biochemical markers are routinely employed to assess the BM iron status. Among the automated cell counter parameters, increase in the RDW is the earliest evidence of iron deficiency. Hb level, RBC count, PCV, MCV, MCH and MCHC values fall progressively with time.

Our study was commenced with an intent to evaluate the Bone Marrow (BM) iron status in different haematological disorders and to correlate it with the peripheral blood red cell indices.

MATERIALS AND METHODS

BM aspirations of 100 patients were done. The Perls' stained smears were evaluated and graded for BM iron stores. Using ABX Pentra 60 automatic cell analyser, RBC indices were determined from the peripheral blood samples.

RESULTS

Significant and fair correlations of MCV (r_s = 0.317, n=100, p<0.05), MCH (r_s = 0.327, n=100, p<0.05), MCHC (r_s = 0.343, n=100, p<0.05) and a significant negative, but fair correlation of RDW-CV% (r_s= -0.292, n=100, p<0.05) with the BM iron grade were observed in our study, which were comparable with similar other studies. MCV had a significant and very good correlation with MCH (r_s = 0.945). 75% of patients with a depleted BM iron store also had simultaneously low MCV and MCH values. Among the RBC indices, MCHC had the strongest correlation (r_s = 0.343) with the BM iron store. 75.7% of the patients with a normal MCHC value also had a normal MCV value.

CONCLUSION

The BM iron grade determined on the Perls' stained aspiration smears and the red cell indices determined by the automatic cell analyser fairly correlated with each other. Therefore, we conclude that the red cell indices may be of use as an affordable, ubiquitous parameter for predicting BM iron stores.

KEYWORDS

Iron Deficiency Anaemia, Perls' Stain, BM Iron Stores, RBC Indices, Surrogate Marker.

HOW TO CITE THIS ARTICLE: Sharma GL, Kumar LD, Singh NB, et al. Bone marrow iron store in Perls' stained aspirates and the red cell indices. J. Evid. Based Med. Healthc. 2017; 4(64), 3830-3835. DOI: 10.18410/jebmh/2017/765

BACKGROUND

Nutritional anaemia, particularly iron deficiency, continues to be a major public health problem in the developing nations. The third National Family Health Survey (NFHS-3).[1] estimates an alarmingly high prevalence of anaemia in India, the prevalence of which in Manipur being 35.7 in women and 11.4 in men.

Evaluation of the depletion of BM iron stores is a sensitive and reliable means for diagnosing Iron Deficiency Anaemia (IDA) even in its pre-latent phase. BM iron, readily identified by Perls' Prussian blue reaction is regarded as a Gold standard for the assessment of BM iron stores, the quantification of which is done using the Gale's grading system.[2]

Combinations of surrogate serum markers, including ferritin, although relatively expensive are routinely employed to assess the iron status of an individual. However, ferritin being an acute phase response protein renders the interpretation of its value and hence the BM iron stores difficult in certain inflammatory conditions.[3]

Financial or Other, Competing Interest: None.
Submission 18-07-2017, Peer Review 25-07-2017,
Acceptance 01-08-2017, Published 08-08-2017.
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DOI: 10.18410/jebmh/2017/765

With most automated full blood counters, the earliest evidence of iron deficiency is an increase in the Red Cell Distribution Width (RDW), which is indicative of the anisocytosis preceding the anaemia. Subsequent events to follow are reduction in the values of haemoglobin, Red Blood Cell (RBC) count, Packed Cell Volume (PCV), Mean Cell Volume (MCV) and Mean Cell Haemoglobin (MCH).

The current study therefore envisages to evaluate the BM iron status using Perls' staining along with an appropriate grading system and its correlation with the various parameters in the RBC indices of the peripheral blood of patients.

Aim and Objectives

Our study was commenced with an intent to evaluate the Bone Marrow (BM) iron status in different haematological disorders and to correlate it with the peripheral blood red cell indices.

MATERIALS AND METHODS

The study was a cross-sectional study conducted in the Department of Pathology, Regional Institute of Medical Sciences (RIMS), Imphal, and was commenced after approval from the Institutional Ethics Committee (IEC), RIMS.

Patients with anaemia and other haematological disorders, having indication for BM aspiration study, irrespective of age, sex, religion and socioeconomic status attending the OPD or those admitted in the wards of RIMS Hospital during November 2013 to August 2015 were included after obtaining prior informed consents. Already aspirated and diagnosed cases were excluded. Since, the study was a hospital-based one and assuming it to be a medium scale study, a minimum of 100 cases were included.

The various study variables were measured and compared, including- Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), BM iron grade and stores.[4]

All patients were subjected to examination of their complete peripheral blood count and bone marrow aspiration study. Using HORIBA ABX Pentra 60 automatic blood analyser of our department, Hb and the RBC indices of the peripheral blood were measured and recorded.

Bone marrow aspiration[5] was done using Salah bone marrow aspiration needle and Perls' staining[5] for BM iron was done on the air-dried films, a control slide always being stained alongside. On the stained BM aspiration smears, haemosiderin is rendered blue and the marrow particles were assessed under the microscope. Each case was assigned an appropriate BM iron grade (Gale's grading) and the corresponding BM iron store of depleted, normal or increased was interpreted (Table 1). Spearman's rank correlation and significance testing were carried out using IBM SPSS V21 software.

Statistical Analysis

Spearman's rank correlation and significance testing were carried out using IBM SPSS V21 software.

RESULTS

Out of the 100 cases, 45% of the patients were males and 55% were females with male-to-female ratio of 1:1.2. Male and female patients with blood haemoglobin levels less than 13 and 12 g/dL respectively were taken as having anaemia.[1] Although, female patients outnumbered males, the proportion of anaemic male patients (88.9%) was more than their female counterparts (87.3%). Only 10.0% of the anaemic males had depleted stores, however, anaemic females had a higher proportion of depleted iron stores (22.9%) (Figure 1).

Each RBC index was compared with the BM iron stores. Maximum proportion of patients having a depleted iron stores (75%) also had low MCV values (<80.9 fL). As with the MCV, most of the patients with a depleted iron store (81.3%) also had a low MCH value (<27 pg) and none of them had high MCH value (>32 pg). A similar trend was also observed with the MCHC values when compared with their respective BM iron store. Most of the iron store depleted patients (56.3%) had a low MCHC value (<31.5 g/dL) and none had high MCHC value (>34.5 g/dL). A different relation was however noted with the RDW values; maximum proportion (75.0%) of the iron store depleted patients had high RDW values (>14.0%) while only 25% of them had normal values. We further found that in 16 patients with depleted BM iron stores, the mean RDW value was 17.9% with a SD of 3.8.

Now each RBC index was correlated with the BM iron grade using the Spearman's rank correlation. MCV, MCH and MCHC had fair correlations (r_s = 0.317, 0.327 and 0.343, respectively) with BM iron grade and this finding is found to be statistically significant (p = 0.001 in all the three). RDW had a negative correlation (r_s = -0.292) with BM iron grade and this finding is found to be statistically significant (p = 0.003). MCV had a very good correlation (r_s = 0.945) with MCH and this finding is found to be statistically significant (p = 0.000) (Figure 2).

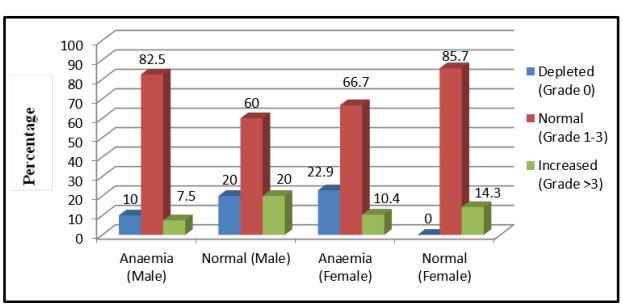

Figure 1. Bar Diagram Showing Relation of Anaemia in Males and Females with BM Iron Store

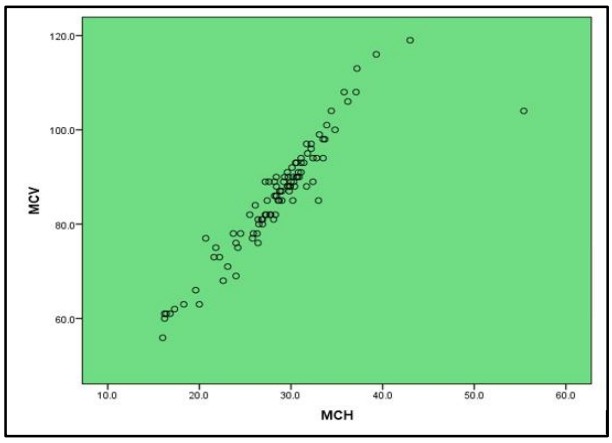

Figure 2. Scattered Diagram Showing Relation between MCV and MCH- Intense Clumping of Dots

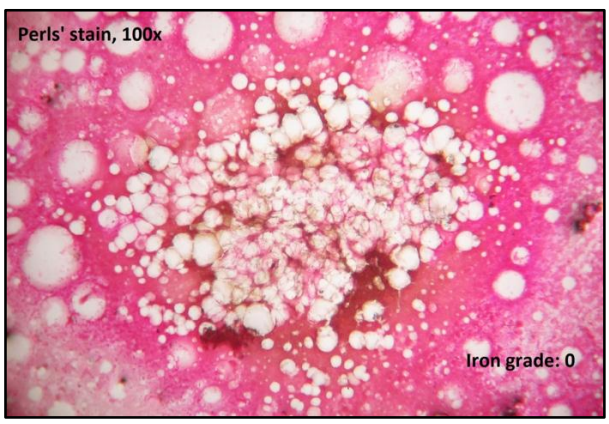

Figure 3. Photomicrograph of Bone Marrow Smear Showing Iron Grade-0 (Perls' Stain, 100x)

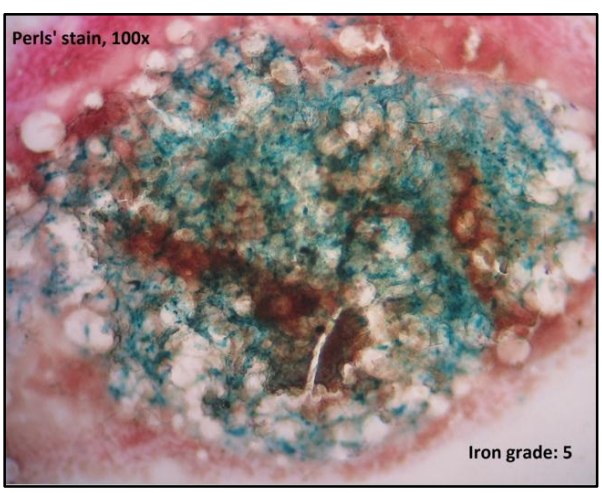

Figure 4. Photomicrograph of Bone Marrow Smear Showing Iron Grade-5 (Perls' Stain, 100x)

DISCUSSION

According to National Family Heath Survey 3 (NFHS-3).[1] The prevalence of anaemia in India is 55.3% in women and 24.2% in men (lower cut-off values of Hb 12 and 13 g/dL in women and men, respectively). Manipur is amongst the few states where the prevalence of anaemia for both women and men is the lowest, however, even in this state, one third of women are anaemic.

In our study, the proportion of anaemic female and male patients were 87.3% and 88.9% respectively with similar cut-off values for Hb.

Out of the 45 male patients, 88.9% (40 of 45) of cases were anaemic (Hb <13 g/dL); a majority of 82% (33 of 40) of these anaemic patients had normal BM iron stores. Similarly, out of the 55 female patients, 87.3% (48 of 55) of cases were anaemic (Hb <12 g/dL); a majority of 66.7% (32 of 48) of these anaemic patients had normal BM iron stores. Also, anaemic patients having depleted (Grade 0; Figure 3) BM iron stores were 10% (4 of 40) and 22.9% (11 of 48) in males and females, respectively. This maybe because of the different BM iron storage patterns in the various aetiological types of anaemia constituting this category. It may also partly be because of the contribution by the cases of early stages of iron deficiency anaemia where the BM iron store may have been reduced, but not yet exhausted.

Bone marrow aspiration is a painful and invasive procedure and is also at times not without associated complications. We therefore thought of a hypothesis that whether routine peripheral blood RBC indices would correlate with the BM iron grade and hence reliably predict the BM iron stores in patients and thus conducted this study of iron staining on the BM aspiration smears and correlation of the iron grade with the peripheral blood RBC indices.

MCV defines the size of the red blood cells and is expressed in femtoliters.[6] The normal value for MCV is of the range 81-101 fL.[7] In our study, among the patients with a low MCV (<80.9 fL), a majority of 75% had depleted BM iron store. This is reiterated by our finding that MCV had a significant and fair correlation with the BM iron store (r_s = 0.317, n=100, p<0.05). The correlation between MCH and BM iron store was similar (r_s = 0.327, n=100, p<0.05). This finding is comparable with the study conducted by Lynn KL et al[8] who found a significant correlation between MCV and serum ferritin concentration and hence with the BM iron stores (r = 0.529, n=131, p=<0001). They also found a significant correlation between MCH with the serum ferritin concentration and hence with the BM iron stores (r = 0.550, n=131, p=<0.001).

In the present study, MCV had a significant, very good correlation (r_s = 0.945, n=100, p<0.05) with MCH (Figure 2). This is in agreement with the study conducted by Fairbanks VF et al[9,10] who found that MCV and MCH almost always correlate with each other closely.

In our study, 75% of patients with a depleted BM iron store had simultaneously low MCV and MCH values. This is comparable with the study conducted by Abdelgader et al[11] who found 66.6% of iron deficient mothers with reduction in two or more of the haematological indices (MCV, MCH and MCHC). They further stated that a combined reduction of MCV and MCH is more sensitive to detect iron deficiency anaemia.

MCH quantifies the amount of haemoglobin per red blood cell.[6] The normal values for MCH range from 27-32 picograms (pg) per cell.[7] In the present study, 81.3% of patients with a low MCH value (<27 pg) also had depleted BM iron store and none of them had a high MCH value (>32 pg). This is supported by our finding that MCH had a significant and fair correlation (r_s = 0.327, n=100, p<0.05)

with BM iron grade. This is comparable with the study conducted by Junca J et al[12] who found that a low MCH of best cut-off point <28 pg was the best predictive parameter of iron deficiency. They stated that the odds ratio for the risk of iron deficiency with a low MCH was 16.5 (95% confidence interval 1.69 to 159.75).

MCHC indicates the amount of haemoglobin per unit volume, which in contrast to MCH correlates to the haemoglobin content with the volume of the cell. It is expressed as g/dL of red blood cells.[6] The normal values for MCHC range from 31.5-34.5 g/dL.[7] In our study, we found that 56.3% of the iron store depleted patients had a low MCHC value (<31.5 g/dL) and none had a high MCHC value (>34.5 g/dL). This is reiterated by our finding that MCHC had a significant and fair correlation (r_s = 0.343, n=100, p<0.05) with BM iron grade. It is worth mentioning here that in our study, among the RBC indices, MCHC has the strongest correlation (r_s = 0.343) with the BM iron store. This is comparable with the studies conducted by Conrad M et al[13] and Klee GG et al[14] who found that a reduced value of MCHC is observed most often in association with iron deficiency and that this index tends to be the last to fall as iron deficiency worsens. We further found that a majority of 75.7% of the patients with a normal MCHC value (31.5-34.5 g/dL) also had a normal MCV value (81-101 fL). This is comparable with the studies conducted by Hershko C et al[15] and Fairbanks VF et al[9] who found that the MCHC value is rarely abnormal when the MCV is normal.

RDW represents the coefficient of variation of the red blood cell volume distribution (size) and is expressed as a percentage.[6] The normal values for RDW range from 11.6-14%.[7] In the present study, we found that a maximum proportion of 75.0% of the BM iron store depleted patients also had high RDW values (>14.0%). This is also supported by our finding of a significant negative, but fair correlation (r_s = -0.292, n=100, p<0.05) of RDW with BM iron store. This is in compliance with the fact that an increased RDW value is an early and pronounced finding in iron deficiency wherein its value may become abnormal even before the MCV falls below the lower limits of normal.[16,17] Our finding of the mean RDW value (CV%) being 17.9% with a SD of 3.8 in the group of patients with depleted BM iron stores is comparable with the study conducted by Alukah R et al[18] who also found the mean RDW value (CV%) of 18.37% with a SD of 2.22 in their group of patients with iron deficiency anaemia.

In all the related previous studies (Table 4), either serum ferritin, serum iron or serum transferrin receptor values were studied for its use as a surrogate for the BM iron store determined by aspiration and grading of the Perls' stained smears. Each of these studies also found a significant correlation between their surrogate parameters and the BM iron store. At least one study conducted by Tiwari M et al[19] compared the RBC indices with serum ferritin level, but not directly with the BM iron grade.

CONCLUSION

In a developing country like India with high prevalence of subclinical iron deficiency, serum ferritin and other related serum biochemical markers are too expensive to be done in all the suspected cases of iron deficiency. In this study, we attempted to find probable, inexpensive surrogate markers amongst our routine laboratory blood parameters, which may be used for reliably predicting BM iron stores and hence iron deficiency. We found significant and fair correlations of MCV (r_s = 0.317, n=100, p<0.05), MCH (r_s = 0.327, n=100, p<0.05), MCHC (r_s = 0.343, n=100, p<0.05) and a significant, negative, but fair correlation of RDW-CV% (r_s = -0.292, n=100, p<0.05) with the BM iron grade, respectively. The pattern of red cell indices in a patient may be of use as an affordable, ubiquitous parameter for predicting BM iron stores.

Microscopic Findings	BM Iron Grade	BM Iron Store
No iron granules seen (Figure 3)	0	Depleted
Small granules in reticulum cells only under oil immersion	1	Normal
Few small granules visible with low power lens	2	
Numerous small granules in all marrow particles	3	
Large granules in small clumps	4	Increased
Dense large clumps of granules (Figure 5)	5	
Very large deposits obscuring the marrow cells	6	

Table 1. BM Iron Grade (Gale's Grading)[2] and the Corresponding BM Iron Store

BM Iron Store	Males			Females		
	Anaemia (%)	Normal (%)	Total (%)	Anaemia (%)	Normal (%)	Total (%)
Depleted Grade 0)	4 (10.0)	1 (20)	5 (11.1)	11 (22.9)	0 (0.0)	11 (20.0)
Normal (Grade 1-3)	33 (82.5)	3 (60)	36 (80.0)	32 (66.7)	6 (85.7)	38 (69.1)
Increased (Grade >3)	3 (7.5)	1 (20)	4 (8.9)	5 (10.4)	1 (14.3)	6 (10.9)
Total	40 (88.9)	5 (11.1)	45 (100)	48 (87.3)	7 (12.7)	55 (100)

Table 2. Corresponding Data for Figure 1

Sl. No.	BM No.	MCV	MCH	Sl. No.	BM No.	MCV	MCH
1.	27/15	92	30.1	51.	30/15	80	26.1
2.	38/14	97	32.2	52.	31/15	84	30.5
3.	27/14	80	26.9	53.	37/15	93	35.8
4.	40/14	76	24	54.	39/15	108	28.7
5.	42/14	99	33.1	55.	44/15	85	28.8
6.	45/14	91	30.8	56.	45/15	87	29.7
7.	46/14	55.9	16	57.	46/15	88	29
8.	47/14	106	36.2	58.	47/15	87	28.4
9.	50/14	73	22.2	59.	48/15	88	31.8
10.	51/14	77	20.7	60.	74/15	95	28.3
11.	55/14	85	27.4	61.	51/15	82	26.9
12.	57/14	89	27.6	62.	52/15	81	25.5
13.	52/14	90	30.2	63.	55/15	82	34.8
14.	53/14	93	30.6	64.	57/15	100	19.6
15.	60/14	78	26.3	65.	59/15	66	34.4
16.	62/14	86	28.4	66.	66/15	104	17.3
17.	65/14	90	30.9	67.	67/15	62	23.1
18.	166/14	104	55.4	68.	68/15	71	30.4
19.	170/14	98	33.7	69.	69/15	88	27.7
20.	29/15	88	29.9	70.	56/15	82	31.1
21.	175/14	81	26.8	71.	60/15	93	30.7
22.	176/14	93	31.4	72.	61/15	90	29.6
23.	177/14	89	28.2	73.	64/15	91	33.5
24.	179/14	89	30.3	74.	70/15	98	33.5
25.	185/14	86	28.4	75.	71/15	94	43
26.	187/14	113	37.2	76.	72/15	119	31.7
27.	189/14	108	37.1	77.	40/15	88	29.3
28.	1/015	87	29.8	78.	49/50	90	29.9
29.	2/015	94	31.1	79.	122/15	88	32.4
30.	10/015	89	30	80.	75/15	89	16.2
31.	11/015	89	29.2	81.	77/15	61	21.6
32.	12/015	60	16.2	82.	78/15	73	32.2
33.	18/015	116	39.3	83.	84/15	96	21.8
34.	19/015	90	28.4	84.	85/15	75	24.2
35.	21/015	101	33.9	85.	86/15	75	30.2
36.	33/15	61	16.4	86.	87/15	85	27.3
37.	35/15	78	23.7	87.	93/15	82	22.6
38.	36/15	88	29.6	88.	94/15	68	32.4
39.	38/15	82	27.2	89.	97/15	94	28.1
40.	73/15	85	33	90.	98/15	81	18.3
41.	158/14	85	28.6	91.	101/15	63	24.5
42.	159/14	87	28.8	92.	103/15	78	26.4
43.	160/14	89	27.2	93.	104/15	76	29
44.	162/14	86	28.2	94.	108/15	85	20
45.	163/14	81	26.4	95.	109/15	63	24
46.	20/15	90	29.7	96.	110/15	69	32.8
47.	22/15	82	27.8	97.	115/15	94	30.6
48.	35/14	97	31.7	98.	116/15	90	25.8
49.	28/15	91	31.1	99.	119/15	77	25.9
50.	172/14	81	26.5	100.	76/15	78	16.8

Table 3. Corresponding Data for Figure 2

Present study	Graded BM iron and stratified the iron store. Found the BM iron grade to have a significant and fair correlation with the peripheral blood RBC indices.
Bableshwar RS et al[3]	Found significant correlation of BM iron store with mean log serum ferritin concentration.
Tiwari M et al[19]	Found significant correlation between serum ferritin concentration and Hb and RBC indices in second and third trimester ANC cases.
Phiri KS et al[20]	Employed an intensive BM iron grading method to assess iron store and found significant correlation between it and soluble transferrin receptor concentration.
Ali MAM et al[21]	Found significant correlation between BM iron store and median serum ferritin value.
Tripathi A et al[22]	Assessed BM iron grade in anaemic patients and found significant correlation of iron store with serum ferritin and iron.

Table 4. Comparison of the Present Study with Previous other Studies

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