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Assessment of Correlation Between the Serum Ferritin Levels and Degree of Iron Stores in Perl's-Stained Slides of Bone Marrow Aspirate

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Abstract

Background: Anemia is a condition in which hemoglobin concentration and/or red blood cell numbers are lower than normal range and insufficient to meet an individual's physiological needs. The present study was conducted to assess correlation between the s. ferritin levels and degree of iron stores in Perl's stained slides of bone marrow aspirate.**Material and Methods:**75 cases of anemia of either gender were enrolled. Peripheral blood films (PBF) were obtained in cases of low hemoglobin. Tissue sections when treated with hydrochloric acid, denature the protein binding to hemosiderin molecules, thereby releasing ferric 3+ ions. The ferric ions combine with potassium ferocyanide to form ferric ferrocyanide which as an insoluble bright blue pigment (Prussian blue). Serum ferritin levels were analysed on the day before, or on the day of bone marrow aspiration by using standardized chemical kit (Calbiotech) based on ELISA technique.**Results:**The mean S. ferritin was 9.75 ± 1.06 in male patients with absent B.M. iron stores. The mean S. ferritin was 8.40 ± 0.93 in female patients with absent B.M. iron stores. The mean Hb 0f 5.72 ± 1.37 SD was seen in iron deficiency and Hb of 7.66 ± 1.86 was seen in iron sufficiency states. The mean S. ferritin of 9.02± 0.69 ng/ml was seen in absent BM iron stores group. Out of 54, 8 patients were reported to have raised and normal serum ferritin as compare to rest of patients of group 0, later on out of 8, 3 were diagnosed with acute leukemia, 1 with multiple myeloma, 1 with lymphoma, 3 were having organomegaly like hepatomegaly and splenomegaly etc. Mean S. ferritin 67.54 ± 15.13 SD was seen in deficient BM iron stores group. Mean s. ferritin of 158 ± 65.49 SD was seen in normal bone marrow iron store group. There was a correlation between B.M Iron stores with serum ferritin obtained by Spearman's Correlation coefficient which was highly significant.**Conclusion:**There was significant correlation between serum ferritin and bone marrow iron stores. Hence clinician should include serum ferritin along with routine investigations of IDA for accurate diagnosis and treatment and proper follow up. Moreover, serum ferritin analysis is non-invasive, convenient and reliable.

Keywords: Bone Marrow Iron, Prussian Blue, Serum Ferritin.

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Introduction

Anemia—a condition in which hemoglobin concentration and/or red blood cell numbers are lower than normal range and insufficient to meet an individual's physiological needs. IDA affects roughly the world's one-third population. Anemia is associated with increased morbidity and mortality in women and children, poor birth outcomes, decreased work productivity in adults and impaired cognitive and behavioral development in children.[1]

For developing effective interventions, understanding anemia's varied and complex etiology is very important that also address the specific causes of anemia. As in India, IDA is most common and prevalent anemia.[2] It is one of the major causes of maternal and child mortality, low physical performance and referrals to healthcare professionals. In population of Punjab, major causes of anemia are various nutritional deficiency like vitamin B12, folate, poor intake etc. Other contributing factors are strict vegetarian diet, parasitic infestations, various infections like malaria, TB and other hemoglobinopathies etc.[3]

Patients can have one or more combinations of above iron deficiency states that all result in iron-restricted erythropoiesis.[4] As ferrtin is one of major storage protein for iron. It is intracellular protein that stores iron and releases it in controlled fashion. It is recommended by WHO to assess iron status using serum ferritin or soluble transferrin receptor. Serum ferritin, is a measure of body storage iron and a sensitive measure of ID.[5] The present study was conducted to assess correlation between the s. ferritin levels and degree of iron stores in Perl's stained slides of bone marrow aspirate.

Materials and Methods

The present study was conducted on 75 cases of anemia reported to department of pathology in collaboration with department of biochemistry, Government Medical College and Rajindra Hospital, Patiala, Punjab. Inclusion criteria was subjects suffering from anemia with hemoglobin below the lower limit of reference level. Subjects from both sexes and from rural and urban communities were included. Exclusion criteria was patients with malignancy, patients on chemotherapy or radiotherapy and patients who refuses to give consent.

Reference level for hemoglobin are given as Men - 15.0 ± 2.0 g/dl and Women - 13.5 ± 1.5 g/dl. The consent was obtained from all enrolled patients. Data such as name, age, gender etc. was recorded.

Peripheral blood films (PBF) were obtained in cases of low hemoglobin. Tissue sections when treated with hydrochloric acid, denature the protein binding to hemosiderin molecules, thereby releasing ferric 3+ ions. The ferric ions combine with potassium ferocyanide to form ferric ferrocyanide which as an insoluble bright blue pigment (Prussian blue). Serum ferritin levels were analysed on the day before, or on the day of bone marrow aspiration by using standardized chemical kit (Calbiotech) based on ELISA technique. Data thus obtained were subjected to statistical analysis. P value < 0.05 was considered significant.

Results

Table 1: Comparison of mean S. Ferritin with respect to gender for subjects with absent B.M. Iron stores

	Mean	S.D.	SEM#	Range	P value	S*
Male	9.75	4.85	1.06	3.1-19.23		
Female	8.40	4.64	0.93	2.9-18.81	0.343	NS

[Table 1] showed comparison of Mean Serum Ferritin to gender in patients with absent B.M. iron stores. The mean S. ferritin was 9.75 ± 1.06 in male patients with absent B.M. iron stores. The mean S. ferritin was 8.40 ± 0.93 in female patients with absent B.M. iron stores.

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The mean S. ferritin was higher in male patients as compare to female patients with p value 0.343 which was non- significant.

Table 2: Comparison of Iron deficiency and sufficiency (deficient and sufficient B.M. Iron stores) states with HB

	Mean Hb	S.D	Range	P value	S*
Iron Deficiency	5.72	1.37	2.90– 558.0	<0.001	HS
Iron Sufficiency	7.66	1.86	94.12– 800.0		

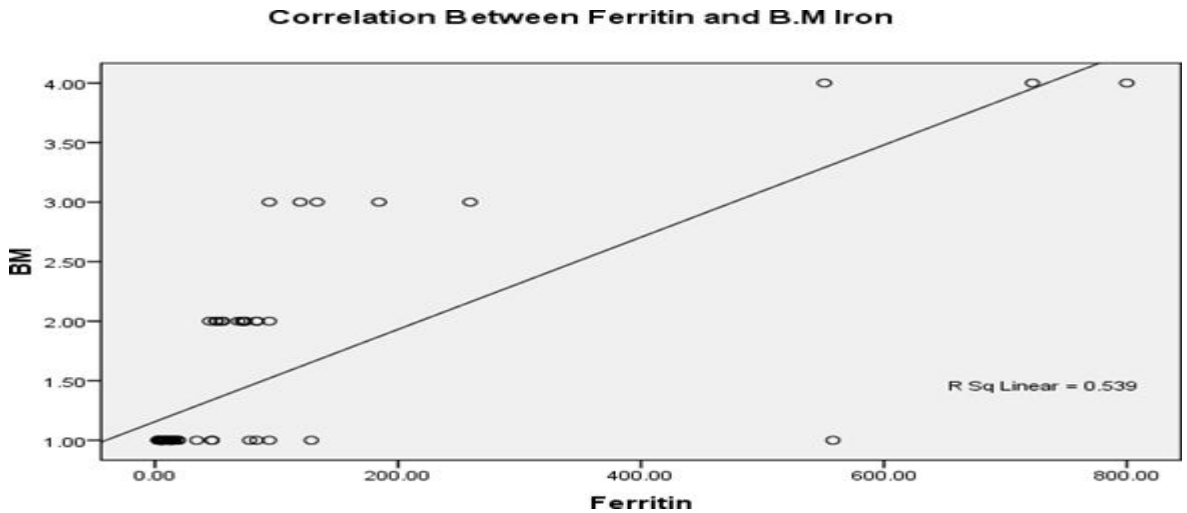
[Table 2] showed comparison of mean Hemoglobin in Iron deficiency and Iron sufficiency (B.M. Iron stores). Mean Hb 0f 5.72 ± 1.37 SD was seen in iron deficiency and Hb of 7.66 ± 1.86 was seen in iron sufficiency states. P value of < 0.001 was obtained and was showing high significance.

Table 3: Comparison of B.M Iron stores with Mean S. Ferritin

Perl's stain Grading	B.M. Ironstores	Mean S.Ferritin	S.D	SEM#	Range	P value	S*
0	B.M Iron Absent	9.02	4.74	0.69	2.90–19.23	<0.001	HS
1+	Deficient B.MIron	67.54	15.13	4.198	45.03–94.12		
2+,3+	NormalB.M Iron	158.14	65.49	29.29	94.12–259.40		
4+,5+,6+	Raised B.M Iron	691.10	127.40	73.55	551.00 –800.00		

Table 4: Spearman's Correlation coefficient with B.M Iron stores with other factors

Factors	R	R2	P value
Serum Ferritin	0.701	0.539	<0.001



Graph 1: Spearman's Correlation coefficient with B.M Iron stores with Ferritin

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[Table 3] showed the comparison of bone marrow iron stores with mean serum ferritin in respective bone marrow iron store groups. Mean S. ferritin of 9.02± 0.69 ng/ml was seen in absent BM iron stores group. Out of 54, 8 patients were reported to have raised and normal serum ferritin as compare to rest of patients of group 0, later on out of 8, 3 were diagnosed with acute leukemia, 1 with multiple myeloma, 1 with lymphoma, 3 were having organomegaly like hepatomegaly and splenomegaly etc. Mean S. ferritin 67.54 ± 15.13 SD was seen in deficient BM iron stores group. Mean s. ferritin of 158 ± 65.49 SD was seen in normal bone marrow iron store group.

[Table 4, Graph 1] showed spearman's correlation of B.M. iron stores with various red cell parameters and serum ferritin. It showed r value of 0.701 and p value of <0.001 in correlation between B.M Iron stores with serum ferritin obtained by Spearman's Correlation coefficient which was highly significant.

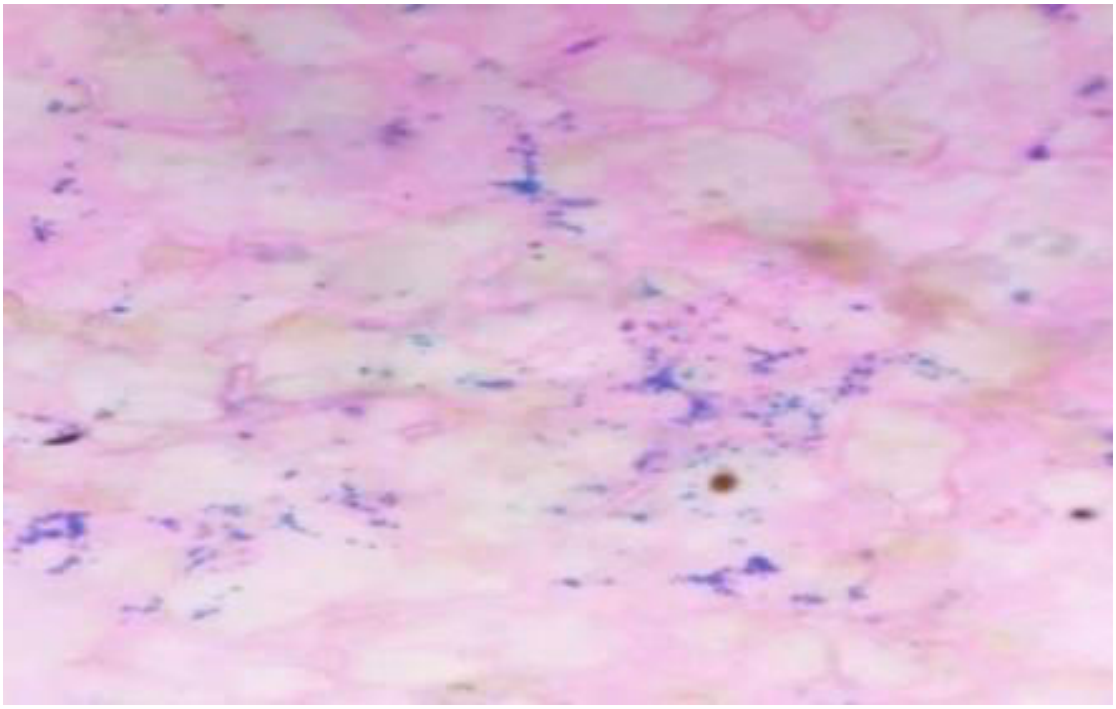

Figure 1: Perl's stained BMA film with iron grade 2+ (100X)

Discussion

India carries the highest burden of anemia despite having an anaemia control programme for 50 years. Among anemia due to nutritional deiciencies; iron deficiency anemia is more prevalent.[6] IDA in India has decreased by only 3.5 percentage points (from 56.5% in 2005–2006 to 53.0% in 2015–2016) between 2005–2006 and 2015–2016, for women aged 15–49 years. During the same period IDA increased in eight states: Delhi, Haryana, Himachal Pradesh, Kerala, Meghalaya, Tamil Nadu, Punjab and Uttar Pradesh, as compared to 27 states. There are numerous causes responsible for anemia most common being deficiency of essential elements for the synthesis of hemoglobin (Iron, Vitamin B12 and Folic Acid), blood loss, repeated pregnancies in females of reproductive age, worm infestation, hemolysis due to known or unknown causes and bone marrow conditions causing suppression of red cell synthesis.[7] Many studies have reflected the high burden of anaemia among women in India consequently leading to risk of low birth weight, neonatal mortality, lower physical and mental activity, reduced working capacity and fatigue.[8,9] Iron deficiency anemia is a major nutritional problem in India and many other developing countries. Iron deficiency anaemia is a major cause of nutritional anaemia in India.[10] Present study was conducted to detect

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correlation between serum ferritin and Bone marrow iron stores in cases of IDA. Samples of serum ferritin was collected and Prussian blue staining of bone marrow aspiration films was done for assessment of stored iron availability for efficient red cell production.

We observed that the mean S. ferritin was 9.75 ± 1.06 in male patients with absent B.M. iron stores. The mean S. ferritin was 8.40 ± 0.93 in female patients with absent B.M. iron stores. The mean S. ferritin was higher in male patients as compare to female patients. In a randomized clinical trial of intravenous (IV) iron therapy in 90 premenopausal nonanemic women presenting with fatigue, serum ferritin ≤50 ng/mL and Hgb ≥ 12 g/dL, fatigue scopes decreased significantly within 6 weeks of IV iron therapy compared to placebo, particularly in women with baseline serum ferritin ≤15 ng/mL.[11]

We found that mean Hb 0f 5.72 ± 1.37 SD was seen in iron deficiency and Hb of 7.66 ± 1.86 was seen in iron sufficiency states. P value of < 0.001 was obtained and was showing high significance. Blend et al,[12] found that serum ferritin levels between 20 and 90 ng/ml correspond with decreased or slightly decreased stores of iron in bone marrow is in concordance with present study's iron deficiency.

We found that mean S. ferritin of 9.02± 0.69 ng/ml was seen in absent BM iron stores group. Out of 54, 8 patients were reported to have raised and normal serum ferritin as compare to rest of patients of group 0, later on out of 8, 3 were diagnosed with acute leukemia, 1 with multiple myeloma, 1 with lymphoma, 3 were having organomegaly like hepatomegaly and splenomegaly etc. Mean S. ferritin 67.54 ± 15.13 SD was seen in deficient BM iron stores group. Mean s. ferritin of 158 ± 65.49 SD was seen in normal bone marrow iron store group.

We found r value of 0.701 and p value of <0.001 in correlation between B.M Iron stores with serum ferritin obtained by Spearman's Correlation coefficient which was highly significant. Present study demonstrated the usefulness of serum ferritin by showing a statistically and graphically significant linear correlation between the serum ferritin and bone marrow iron stores.

Conclusion

Authors found that there was significant correlation between serum ferritin and bone marrow iron stores. Hence clinician should include serum ferritin along with routine investigations of IDA for accurate diagnosis and treatment and proper follow up. Moreover, serum ferritin analysis is non-invasive, convenient and reliable.

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