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CORRELATION STUDY BETWEEN BONE MARROW IRON AND SERUM IRON AND SERUM FERRITIN IN PATIENTS OF MODERATE TO SEVERE ANEMIA

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ABSTRACT: BACKGROUND: Iron deficiency anemia is one of the most prevalent forms of malnutrition. The list of diagnostic tools is quite long starting from the basic hemogram, p.s for comment, reticulocyte count to iron studies including serum ferritin, serum iron, total iron binding capacity. Evaluation of the bone marrow iron stores is the gold standard. **AIM:** To perform a correlation study between Perl's Prussian blue stained bone marrow aspirate smears and serum iron and serum ferritin. **MATERIALS AND METHODS:** A descriptive study of Perl's Prussian blue stained bone marrow aspirate smears of 40 adult patients with moderate to severe anemia was performed at the Department of Pathology, Gandhi Medical College, Bhopal. Bone marrow was assessed by Gale's method and correlated with serum ferritin and serum iron. **RESULTS:** Gale's grading method revealed decreased iron stores in 30%; normal iron stores in 55% cases, and 15% cases had increased iron stores. The co-relational coefficient between bone marrow iron grade and serum ferritin came out to be 0.59 with an F- test value of 55.10. The correlation coefficient between bone marrow grade and serum iron is 0.76 and the F-test value -117.7. **CONCLUSION:** Although bone marrow iron grading is still gold standard but there exists a positive correlation between bone marrow iron with serum ferritin and serum iron thus iron status can be assessed by serum ferritin and serum iron, bone marrow being an invasive procedure can be avoided, also it will be helpful where bone marrow examination facility is not available.

KEYWORDS: Bone marrow iron. Gale's method, serum ferritin, serum iron.

INTRODUCTION: World -wide iron deficiency anemia is a major public health problem especially in developing countries. According to NFHS- 3, India report; anemia affects an estimated 50% of the Indian population.¹ The proportion of anemia caused by iron deficiency in females of age group 15-49 years in India is 70-90%.²

A number of markers like serum ferritin, serum iron, total iron binding capacity, and transferrin saturation are used to assess the iron status of the individual,³ but microscopic examination of the stainable iron in the bone marrow is considered the gold standard for determining the body iron stores.³

Ferritin is the intracellular storage form of iron, found chiefly in the cytoplasm of the reticulo-endothelial system.⁴

The concentration of the plasma (or serum) ferritin is positively correlated with the size of the total body iron stores in the absence of inflammation.⁵

Values peak among men between 30-39 years of age and then tend to remain constant until about 70 years of age. Among women, serum ferritin values remain relatively low until menopause

and then rise. In most normal adults serum ferritin concentration lie within the range of 15-300ug/l. In adults concentrations < 15ug/l indicate an absence of storage iron.⁶

A low serum ferritin is highly suggestive of deficient iron stores.

Iron is carried in the plasma bound to the protein transferrin. This molecule binds two atoms of iron as Fe³⁺ and delivers iron to cells by interaction with membrane transferrin receptors. Measurement of the serum iron alone provides little useful clinical information because there is considerable variation from hour to hour and day to day.⁵

Serum ferritin concentrations have been documented to give an accurate indication of the amount of storage iron not only in healthy individuals but also in cases of iron deficiency or iron overload.^{4,5} Values less than 20ug/l indicate negative iron balance or decreased stores.⁵ In patients with acute or chronic diseases it can be up to 100ug/l with an absence of stainable bone marrow iron.⁵

Transferrin saturation is the ratio of serum iron concentration and TIBC expressed as percentage.⁷

The most reliable diagnostic parameter to identify absolute iron deficiency in patients with chronic inflammation is the ratio of serum Transferrin receptor concentration to the log of serum ferritin concentration.⁷

Serum transferrin receptor protein is released by the erythroid precursors in the blood and their levels are increased in iron deficiency anemia which can be measured but needs highly sophisticated technique.³

Cases of iron deficiency anemia need to be differentiated from thalassemias on the basis of serum iron indices. Normal or increased serum iron levels & transferrin saturation are characteristic of the thalassemias.³

One of the most common diagnostic problems encountered by clinicians is the distinction between absolute and functional iron deficiency.³

In absolute or true iron deficiency there is absence or decreased endogenous iron stores where as in functional iron deficiency there is lack of utilization of endogenous iron.^{4,5} It is important to differentiate between the two from the treatment point of view.

Conventionally iron has been primarily assessed in marrow fragments which represent iron stores in the form of hemosiderin.⁸

The body iron reserve is traditionally assessed by the biochemical markers of iron metabolism, namely serum ferritin, serum iron, total iron binding capacity.

In view of all these different kind of investigations, centres with limited resources serum ferritin and serum iron can be performed and correlated the results with stainable bone marrow iron. Gale's has described a method for bone marrow iron grading which assesses iron in marrow fragments only.^{8,9}

Gale's grading⁷:

- Grade 0 None, No visible iron under high power magnification
- Grade 1 Very slight, Small iron particles just visible in few reticulum cells under high power magnification
- Grade 2 Slight, sparsely distributed iron particles just visible under low power magnification
- Grade 3 Numerous small iron particles present in reticulum cells throughout the marrow fragment

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- Grade 4 Moderate to heavy, Larger iron particles throughout the fragment with tendency to aggregate into clumps
- Grade 5 Heavy, large clumps of iron throughout the fragment
- Grade 6 Very heavy, Very large deposits of iron, both intra- and extra-cellular, obscuring cellular detail in the fragments.

MATERIALS AND METHODS: We conducted the study on 40 patients with moderate to severe anemia, coming for investigations in the Department of Pathology, Gandhi Medical College Bhopal from March to December 2013.

After taking a written informed consent from the patient, bone marrow aspirate was obtained from the sternum observing strict asepsis. The air dried slides were immediately fixed in 90% alcohol for one hour, to ensure proper fixation and they were then stained with Perl's Prussian blue stain (which consist of Potassium ferrocyanide and HCl which reacts with the ferritin in the cells to form a blue color granules). The Gale's grading method was employed to assess iron in the marrow fragments. A minimum 7 bone marrow particles were studied. Interpretation of Perl's stain results according to this grading system-grade 0: iron deficiency; grade 1: diminished iron stores; grade 2 and 3: normal iron stores; grade 4 to 6: increased iron stores.⁷

Blood samples were collected at the same time for serum ferritin and serum iron estimation. Serum ferritin level determined by microparticle enzyme immunoassay. Serum iron levels were estimated colorimetrically.

Bone marrow iron results were compared with serum ferritin and serum iron.

Ethical clearance was taken from institutional ethical committee.

RESULTS: Out of the 40 patients 12 patients got grade1 i.e decreased iron stores (image 1), 22 patients belonged to grade 2 and 3 i.e. normal iron stores (image 2) and 6 patients of grade 4 i.e. increased iron stores (image 3). We used correlation-coefficient to measure the strength and the direction of linear relationship between two variables and then applied F-test to compare two variance or standard deviations. The p-value as we will see in the following tables is <0.001 and is significant.

BM grade	Serum Iron		Serum Ferritin	
	Mean	Standard deviation (S.D.)	Mean	SD
Grade 1 (decreased)	19	7.4	21.8	5.8
Grade 2, 3 (normal)	53	30.1	48.4	27.2
Grade 4 (increased)	167	52.1	220	115.1

TABLE 1: Distribution of cases according to grade of iron and serum iron & serum ferritin

As the above table is showing there were 12 patients who had decreased marrow iron stores, belonged to grade 1. Serum iron and ferritin levels of these patients were measured and their mean and (S.D.) were 19(7.4) and 21.8(5.8) respectively.

In the next grade, there were 22 patients having normal bone marrow iron i.e. they belonged to grade 2 and 3. The mean and S.D. values of serum iron and ferritin were 53(30.1) and 48.4(27.2) respectively.

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In grade 4, there were 6 patients with increased iron stores. The mean and S.D. values of serum iron and ferritin were 167(52.1) and 220 (115.1) respectively.

The following table shows correlation between bone marrow iron grading and serum ferritin as the correlation- coefficient is more than 0.5, it shows a positive correlation.

Bone marrow iron grade	Serum ferritin
Correlation coefficient	0.59
F-test	55.10
p-value	<0.0001significant.
TABLE 2	

Table showing correlation between bone marrow iron grading and serum iron as the correlation coefficient is more than 0.5 and close to +1, It shows a positive correlation.

Bone marrow iron grade	Serum iron
Correlation coefficient	0.76
f-test	117.7
P value	<0.0001 sig
TABLE 3	

Table showing correlation between serum iron and serum ferritin. As the correlation coefficient is more than 0.5 and close to +1, it shows a strong positive correlation.

Serum iron	Serum ferritin
Correlation coefficient	0.81
f-test	162
P-value	<0.0001 sig
TABLE 4	

DISCUSSION:

Study	Iron status	Patient distribution	Ferritin mean log	Ferritin S.D.	Ferritin median ug/l	Ferritin Mean (S.D.)
1. Luxton et al (1978) ¹⁰	Normal	46%	-	-	120	-
	Decreased	27.8%	-	-	7	
	increased	25.4%	-	-	312	
2. Phiri et al (2009) ⁸	Normal	66.2%	2.7	0.5	-	-
	deficiency	33.8%	1.8	0.7	-	-

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3. Bableswhar et al(2013) ¹¹	Normal	37.5%	2.13	0.38	-	-
	functional Iron deficiency	23.75%	2.65	0.24	-	-
	Iron stores deficiency	16.25%	0.91	0.57	-	-
	combined	22.5%	2.04	0.78	-	
4. Present study	Normal	22	-	-	-	48.4(27.2)
	Decreased	12	-	-	-	21.8(5.8)
	Increased	06	-	-	-	220(115.1)

TABLE 5: Following table showing comparison between previous similar studies

There is positive correlation of mean ferritin levels and with the bone marrow iron grading in our study similar to Luxton et al, (1978), Phiri et al and Bableswhar et al.

Luxton et al¹⁰ studied 248 patients, bone marrow iron results and serum ferritin levels were correlated. Patients were divided in three groups –normal, increased and decreased iron stores. These were correlated with median ferritin values. Phiri et al.⁸ employed an intensive method of assessing marrow iron in 303 children (aged 6 to 60 months) assessing iron in marrow fragments, in macrophages around fragments, and in erythroblasts. Fragment and macrophage iron reflect iron stores while iron in the erythroblast is indicative of utilizable iron which is diminished in functional iron deficiency.

Bableswhar et al¹¹ performed a similar study with 40adult patients, in which they graded bone marrow iron with Gale's method as well as with intensive grading method and correlated the results with mean log serum ferritin concentration.

In the present study bone marrow iron was assessed in patients with moderate to severe anemia and grading was done as per the Gale's grading method. Patients were classified in three grades –normal, decreased and increased iron stores. Furthermore, we attempted correlation of the marrow iron results with the serum ferritin and serum iron and got positive correlation between them.

The data reported in this study confirms the usefulness of measuring the serum ferritin and serum iron concentration to assess the body iron stores.

CONCLUSION: There is a positive correlation between bone marrow iron and serum iron & ferritin. In spite of bone marrow iron studies being the gold standard in evaluating iron status of the body, Serum ferritin and serum iron can be used to assess body iron stores. Bone marrow being an invasive procedure can be avoided; also it will be helpful where bone marrow examination facility is not available or not possible.

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Image 1: 10x view: Bone marrow iron grade I

**Image 2: 10x view:
Bone marrow iron grade II**

**Image 3: Scanner view:
Bone marrow iron grade IV**

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