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A Study to Correlate Serum Ferritin, Serum Iron and Serum TIBC Levels with Morphological Grading of Bone Marrow Iron Stores in Anemic Persons

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ABSTRACT
Iron deficiency anemia (IDA) is typically caused by inadequate intake of iron, chronic blood loss, or a combination of both. The IDA is characterized by microcytic hypochromic erythrocytes and low iron stores. Functional iron deficiency (FID) is a state in which there is insufficient iron incorporation into the hemoglobin of erythroid precursors in the face of apparently adequate body iron stores. Bone marrow smears and blood investigation report of patients age more than 18 years and of either sex who are referred to the Department of Pathology for bone marrow aspiration as part of diagnostic work up for anemia were included in this study. In our study, there is a significantly steady increase in serum ferritin with Gale's grade with mean serum ferreting values of 22.2±14.7, 91.4±56.5, 233.3±92.8, 677.7±553.7 and 1191.9±479.3 for grade 0, 1, 2, 3 and 4, respectively. In our study, there was a significant decline in mean serum TIBC levels with Gale's grade with mean serum TIBC values of 407.6±46.8, 335.4±64.7, 257.2±51.9, 229.3±104.2 and 315.4±96.2 for grade 0, 1, 2, 3 and 4, respectively.

INTRODUCTION

Anemia is a major health problem worldwide, especially in developing countries like India. Anemia (Greek, 'anaimia', meaning 'lack of blood') is defined by a decrease in the total amount of hemoglobin (less than 12g/dl in women and 13.5 in men) or the number of red blood cells. Iron deficiency anemia is the most common anemia in the world[1]. Iron deficiency anemia (IDA) is typically caused by inadequate intake of iron, chronic blood loss, or a combination of both. The IDA is characterized by microcytic hypochromic erythrocytes and low iron stores. Functional iron deficiency (FID) is a state in which there is insufficient iron incorporation into the hemoglobin of erythroid precursors in the face of apparently adequate body iron stores. Anemia of chronic disease (ACD) occurs when a persistent inflammatory state leads to the sequestration of iron, reducing iron availability for red cell production[2]. Typically, evaluation of the cause of anemia includes a complete blood cell count, peripheral smear, reticulocyte count and bone marrow examination. Bone marrow iron concentrations have shown a close correlation with serum ferritin (Normal value 24-336µg/L in men and 11-307µg/L in women). A serum ferritin level of 1µg/L is approximately equal to 8 to 10 mg of storage iron[3]. The serum ferritin level may be misleading in the presence of acute or chronic inflammation as serum ferritin is also an acute phase reactant and thus one cannot exclude iron deficiency as the cause of anemia when serum ferritin is normal or even elevated in the presence of inflammatory process. In the presence of underlying infection or inflammation, other iron markers like serum iron and serum TIBC may be useful but, the limitations are that it is not as reliable as ferritin. Serum iron levels (Normal value 70-175µg/L in men and 50-150µg/L in women) are a direct measure of amount of iron bound to transferrin. Serum total iron binding capacity (TIBC) (Normal value 250-450µg/L) is an indirect measurement of protein transferring in terms of the amount of iron it will bind. Serum TIBC is a useful index of nutritional status and discriminates well between biochemically defined IDA and ACD[4]. Bone marrow examination is a critical part of the evaluation of patients with a variety of hematopoietic and non -hematopoietic diseases. It is important to establish, whether the iron deficiency is present or not by demonstrating the absence of stainable iron via bone marrow examination which remains the gold standard for diagnosis. Perls' stain on bone marrow aspirates is an important tool to study iron within sideroblasts, macrophages and erythrocytes and assess iron stores[5]. Where sophisticated instruments and facilities are not available for measuring iron in the body by chemical method, this simple technique is efficient, cost-effective and results are reliable[6]. Bone marrow iron studies help to decide whether iron therapy would be of any use or not in cases of anemia[6]. Hence this study has been taken to correlate serum ferritin, serum iron and serum TIBC levels with morphological grading of bone marrow iron stores and to evaluate serum ferritin as a reliable indicator of bone marrow iron stores in case of iron deficiency anemia and anemia associated with chronic infections, inflammations and malignancy.

MATERIALS AND METHODS

Study Design: Descriptive cross-sectional study.

Study Population: Bone marrow aspirate of patients age >18 years and of either sex, who are referred to the Department of Pathology, as part of diagnostic work up for anemia were included in this study.

Sampling Method: Consecutive samples as per the inclusion and exclusion criteria.

Inclusion Criteria:

- Bone marrow smears and blood investigation report of patients age <18 years and of either sex who are referred to the Department of Pathology for bone marrow aspiration as part of diagnostic work up for anemia were included in this study.

Exclusion Criteria:

- Marrow sample found to be diluted with blood.
- Marrow smears having poor material or inadequate material.
- Marrow samples from those who undergoing blood transfusion one week prior to bone marrow aspiration.
- Marrow samples from those who are on parenteral or oral iron supplementation one week prior to bone marrow aspiration.

Data Collection: Data collection is done by collecting the bone marrow smears, which are obtained from bone marrow aspiration done in Department of pathology. These bone marrow smears will be stained by Perls' stain and assessed microscopically according to Gale's histological grading method and intensive histological grading method for iron stores. The blood investigation reports of complete hemogram, peripheral smear, serum ferritin, serum iron and serum TIBC from blood samples sent to central diagnostic laboratory was collected.

Analysis: The data collected will be entered in excel sheet and analysed using Epi data/SPSS software. Descriptive statistics like frequency, proportions, percentage, mean, standard deviation and inferential statistics like chi square test to know the association, t-test to know the difference between two groups and other relevant tests will also be used as applicable.

Table 1: Mean Level of Serum Ferritin to Bone Marrow Iron Grade

	Gale's histological grading for iron stores					
Serum ferritin	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**P-value**
Range	12-60	23-186	112-335	265-2000	883-2000	**0.001**
Mean	22.2±14.7	91.4±56.5	233.3±92.8	677.7±553.7	1191.9±479.3	
Median	16.3	71.4	265.1	547.2	904.9	

Table 2: Post Hoc Assessment of Serum Ferritin with Gale's Grading

	P value for serum ferritin with Gale's grade				
Gale's grade	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
0	-	0.976	0.256	0.001	0.001
1	0.976	-	0.643	**0.001**	**0.001**
2	0.257	0.643	-	**0.002**	**0.001**
3	**0.001**	**0.001**	**0.002**	**-**	**0.011**
4	**0.001**	**0.001**	**0.001**	**0.011**	**-**

Table 3: Mean Level of Serum Iron to Bone Marrow Iron Grade

Serum iron	Gale's histological grading for iron stores					
	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**P-value**
Range	13-44	13-168	20-120	28-202	73-235	**0.001**
Mean	24.1±10.6	51.8±44.6	62.3±35.7	107.6±68.7	149.4±58.3	
Median	27.0	38.5	50.5	100.5	138.0	

Table 4: Post Hoc Assessment of Serum Iron with Gale's Grading

Gale's grade	P value for serum iron with Gale's grade				
	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
0	-	0.612	0.180	**0.002**	**0.001**
1	0.612	-	0.972	0.068	**0.001**
2	0.180	0.972	-	0.113	**0.002**
3	**0.002**	0.068	0.113	-	0.451
4	**0.001**	**0.001**	**0.002**	0.451	-

Table 5: Mean Level of Total Iron Binding Capacity (TIBC) to Bone Marrow Iron Grade

Serum TIBC	Gale's histological grading for iron stores					
	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**	**P-value**
Range	329-474	254-474	203-364	17-363	204-443	**0.001**
Mean	407.6±46.8	335.4±64.7	257.2±51.9	229.3±104.2	315.4±96.2	
Median	423.0	318.0	245.0	231.0	273.0	

Table 6: Post Hoc Assessment of Serum TIBC with Gale's Grading

Gale's grade	P value for serum TIBC with Gale's grade				
	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
0	-	0.143	0.000	**0.001**	0.116
1	0.143	-	**0.040**	0.016	0.983
2	**0.001**	**0.040**	-	0.865	0.447
3	**0.001**	0.016	0.865	-	0.192
4	0.116	0.983	0.447	0.192	-

Table 7: Mean Level of Serum Ferritin with Different Storage Deficiency by Intensive Grading Method

Serum ferritin	Intensive grading method				
	Normal	**Functional**	**Iron storage**	**Functional and iron storage**	**P-value**
Range	883-2000	265-2000	112-335	12-186	**0.001**
Mean	1191.9±479.2	677.6±553.7	233.3±92.8	56.8±53.6	
Median	904.9	547.2	265.1	32.5	

Table 8: Mean Level of Serum Iron with Different Deficiency by Intensive Grading Method

Serum iron	Intensive grading method				
	Normal	**Functional**	**Iron storage**	**Functional and iron storage**	**P-value**
Range	73-235	28-202	20-120	13-168	**0.001**
Mean	149.4±58.3	107.6±68.7	62.2±35.7	38.0±34.6	
Median	138.0	100.5	50.5	28.0	

Table 9: Mean Level of Serum TIBC with Different Storage Deficiency by Intensive Grading Method

Serum TIBC	Intensive grading method				
	Normal	**Functional**	**Iron storage**	**Functional and iron storage**	**P-value**
Range	204-443	17-363	203-364	254-474	**0.001**
Mean	315.4±96.2	229.3±101.2	257.2±51.9	371.5±66.3	
Median	273	231	245	384	

RESULTS AND DISCUSSION

In our study, there is a significantly steady increase in serum ferritin with Gale's grade with mean serum ferritin values of 22.2±14.7, 91.4±56.5, 233.3±92.8, 677.7±553.7 and 1191.9±479.3 for grade 0, 1, 2, 3 and 4, respectively. This difference was statistically significant (p=0.001). In a study conducted by Ahuja[7] mean it was found that serum ferritin values of 40.09, 184.29, 408.6430, 594.26 and 680.91 for grades 0,1,2,3 and 4 respectively, this result was statistically significant(P<0.001), which was comparable to our study. In our study, there was a significantly steady increase in serum iron with Gale's grade with mean serum iron values of 24.1±10.6, 51.8±44.6, 62.3±35.7, 107.6±68.7and 149.4±58.3 for grade 0, 1, 2, 3 and 4, respectively. This difference was statistically significant (p=0.001). This result was compared with the study conducted by Ahuja[7], showed mean serum iron values of 82.56, 161.43, 96.96, 129 and 201.45 for grades 0, 1, 2, 3 and 4 respectively. A Higher proportion of cases with lower Gale's grade had low serum iron compared to patients with higher Gale's grade. This difference was statistically significant (p=0.001). This finding was similar to the study conducted by Pujara[8] which showed serum iron has a direct correlation with bone marrow iron stores (p=0.127). In our study, there was a significant decline in mean serum TIBC levels with Gale's grade with mean serum TIBC values of 407.6±46.8, 335.4±64.7, 257.2±51.9, 229.3±104.2 and 315.4±96.2 for grade 0, 1, 2, 3 and 4, respectively. This difference was statistically significant (p=0.001). This result was correlated with a study conducted by Ahuja[7], which showed mean serum TIBC values of 377.49, 369.712, 281.66, 270.11 and 231.91 for grades of 0, 1, 2, 3 and 4 respectively. Our study showed a very high correlation (r=0.917, p=0.001) between Gale's iron grading with serum ferritin. The correlation was moderate and positive with serum iron (r=0.662, p=0.001) and moderate and negative with TIBC (r=-0.557, p=0.001). This was comparable to a study done by Ahuja[7], which showed a very high correlation (r=0.865, p <0.001) between Gale's iron grading with serum ferritin. The correlation was positive with serum iron (r=0.239, p=0.016) and moderate and negative with TIBC (r=-0.497, p<0.001).

In our study, there was a significant increase in serum ferritin with bone marrow classification by intensive grade method with increased serum ferritin values in normal patients (1191.9±479.2), compared to functional iron deficiency (677.6±553.7), iron storage deficiency (233.3±92.8) and functional with iron storage deficiency (56.8±53.6), respectively. This difference was statistically significant (p=0.001). This was compared with a study done by Singh[10], which showed mean serum ferritin values in normal patients (213.83-231.6), functional iron deficiency (212.62-258.35), iron store deficiency (39.1-36.76) and functional and iron store deficiency (54.4-178.05) respectively. On further assessment, it was noted that serum ferritin level was significantly higher in normal patients on intensive grade method compared to deficient groups (p<0.05). Furthermore, serum ferritin level was significantly lower in iron storage deficiency group compared to functional iron deficiency group (p=0.001). Similarly, serum ferritin level was significantly lower in a patient with both functional and iron storage deficiency compared to patients with functional iron storage deficiency alone (p=0.001). when compared to the study done by Singh[10], their results showed mean serum ferritin in a correlation between functional iron deficiency and combined deficiency was found to be statistically significant (p=0.002)[9]. In our study when compared with bone marrow iron status classification by intensive grade method, serum iron was significantly higher in normal patients (149.4±58.3), compared to functional deficiency (107.6±68.7), iron storage deficiency (62.2±35.7) and functional with iron storage deficiency (38.0±34.6). Overall, the difference in serum iron levels between the groups was statistically significant (p=0.001). The majority of cases with functional and iron storage disorder had lower serum iron of <50 units compared to patients with iron store deficiency (42.1%) and functional deficiency (25%). Overall, significantly lower serum iron was seen in combined deficiency, followed by iron store deficiency and functional deficiency. This difference was statistically significant (p=0.001). There was a significant difference in serum TIBC level across different diagnoses by iron grade method (p=0.001). The mean serum TIBC in normal, functional deficiency, iron storage deficiency and combined functional with iron storage deficiency were 315.4±96.2, 229.3±101.2, 257.2±51.9 and 371.5±66.3, respectively. Mean TIBC was decreased in functional, whereas normal in iron storage and increased in combined functional and iron storage[10].

CONCLUSION

In this study serum ferritin, serum iron and serum TIBC were correlated with bone marrow iron study using Gale's method of grading in bone marrow aspiration was finally concluded that serum ferritin and serum iron are reliable indicators and can be used as surrogate markers for daily clinical practice in assessing

iron status, as this is a simple non-invasive technique and cost-effective.

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