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CO-RELATION OF SERUM FERRITIN, IRON, TIBC LEVELS WITH MORPHOLOGICAL GRADING OF BONE MARROW IRON STORES IN BONE MARROW ASPIRATION STUDIES

Pathology

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ABSTRACT

BACKGROUND:Measurement of bone marrow iron (hemosiderin) by histochemical Prussian blue/Perl's staining is taken as gold standard to diagnoseiron status/iron overload and to differentiate morphologically similar anemia due to chronic disorder versus nutritional causes.
AIMS:To evaluate dynamics of iron metabolism in various conditions.
STUDY DESIGN: This prospective study was performed in cases who have undergone bone marrow aspiration to access the bone marrow iron stores
SUBJECTS & METHODS: To correlate serum levels of ferritin, iron, total iron binding capacity with morphological bone marrow aspiration iron Gale's grading in 100 consecutivecases.
STATISTICALANALYSIS USED: Chi-square and Spearman correlation tests were done using SPSS 18.0 software (IBM Inc).
RESULTS: Statistical data proved that P value of BM iron grading is < 0.001 with both serum ferritin and TIBC, however overall correlating best with serum ferritin (Spearman correlation 0.865) as compared to TIBC and serum iron.

KEYWORDS

Serum Ferritin, Serum iron, Total iron binding capacity (TIBC), Bone marrow iron store

INTRODUCTION

Iron comprises 5% of the earth's crust and 0.004% of the human body's mass. Iron is an integral part of hemoglobin, myoglobin, cofactor for numerous metabolic reactions and present as trace element in biological systems. Among two iron storage compound i.e. ferritin and hemosiderin, the former is water-soluble and present in plasma in equilibrium with ferritin molecules of histiocytes. Similarly bone marrow contains iron in the form of ferritin and hemosiderin. Most of the non-circulating iron in the RBC's is stored as Fe+++ and in marrow histiocytes as hemosiderin, where it is available to nearby RBC's precursors. Hemosiderin is a more stable and less available form of storage iron.[1]

Bone marrow non-heme iron concentrations have shown close correlations with serum ferritin.[2] Serum ferritin level of 1 µg/L is approximately equal to 8-10 mg of storage iron. In normal adult male and female serum. ferritin ranges from 20-500 µg/L (average 90-95 µg /L) and 12-200 µg/L (average35 µg/L) respectively.[3] Serum ferritin levels of <12 µg/L are taken to be diagnostic of iron deficiency anemia (IDA). Serum ferritin values have been compared with results of tissue non-heme iron concentration in patients with iron stores ranging from deficiency to very elevated levels. A disproportionate rise of serum ferritin concentration is observed in liver diseases, leukemia, lymphoma, hemosiderosis, spherocytosis, end stage renal disease, rheumatoid arthritis, megaloblastic anemia, sideroblastic anemia, alcohol intake, aplastic anemia, acute myocardial infarction and hyperthyroidism. However, falsely low values have been reported in hypothyroidism and vitamin C deficiency, they are seldom of the magnitude to be confused with iron deficiency anemia.[4]

Serum iron levels are a direct measure of amount of iron bound to transferrin. The normal mean serum iron value for men is 120 µg/dl (21.8 µmol/L) with range of 70 to 200 µg/dl (14 to 36 µmol/L).[3] These levels are influenced by the recent ingestion and absorption of iron medication. The levels are increased in hemosiderosis, hemolytic anemia, aplastic anemia, sideroblastic anemia, thalassemia, renal dialysis, multiple transfusions, and oral contraceptive use while decreased in anemia of chronic diseases (ACD), acute inflammation, myocardial infarction, acute collagen diseases, malaria, enteric fever, nephrosis, impaired iron absorption.

TIBC is an indirect measurement of protein transferrin in terms of the amount of iron it will bind. The normal range of TIBC is 250 to 435 µg/dl (average 340 µg/dl = 61 µmol/L).[3] TIBC is a useful index of nutritional status and discriminates well between people with biochemically defined IDA and ACD.[3] A high TIBC or transferrin seen in pregnancy, oral contraceptives use (due to increased production of transferrin by liver), IDA, polycythemia vera. A low TIBC or transferrin may occur in hemochromatosis, ACD and malnutrition.

This study proposes the importance of serum levels of ferritin, iron, and TIBC in various anemias, infections, malignancies and to find out whether these biochemical assays can help to know the dynamics of iron metabolism in these conditions and can be used to eliminate the number of BMA (semi quantitative and subjective method) done purely to know the iron stores.[5]

MATERIALS AND METHODS:

This prospective study was performed from Oct 2007 to Sep 2008,

after due approval by the Command Hospital Air Force Bangalore Ethics Committee. 100 (OPD/admitted) cases of all age groups, both sexes with different diagnoses, who have undergone BMA to access the bone marrow iron stores were included in the study.

Hematological investigations like hemoglobin (Cyanmethemoglobin method), total leukocyte count, platelet count and differential leukocyte count on peripheral blood examination (Leishman's stain) were performed as per standard protocols.[6,7]

Biochemical tests for serum levels of ferritin, iron, TIBC were performed. 4 - 5 ml of venous blood sample from the anterior cubital vein was drawn, which was later separated by centrifugation at room temperature at 1000 rpm for 10 minutes. The desired parameters were measured in the sera within 24 hours of collection or sample was stored at 00C till further analysis. Serum ferritin levels were measured by enzyme immunoassay technique with the steps followed as per the provided kit CALBIOTECH.[8] Serum iron and TIBC were measured by end point reaction using RA50 equipment and the steps were followed as per provided kit "RAICHEM". Serum iron values were read as on RA 50. Serum TIBC was calculated as: UIBC = (500 – value noted as from RA 50) was used to calculate TIBC = Serum Iron + UIBC.

Bone marrow examination was done by Leishman's stain and Prussian blue stain for iron (based on the reaction of hemosiderin i.e. ferric form with potassium ferrocyanide leading to formation of a blue coloured compound ferriferrocyanide).[9] Bone marrow smears were first assessed under low power (10x) to look for bone marrow fragments. These fragments were then assessed for golden yellow refractile granules lying free or in macrophages, according to morphological grading of iron stores.[10] Normal bone marrow iron grade is 1+ to 3+ [Table 1].[3]

Table 1: MORPHOLOGICAL GRADING OF IRON STORES IN BONE MARROW

Grade	Criteria	Iron content µg/gm
0	No iron granules observed	43 + 23
1+	Small granules in reticulum cells seen only with oil immersion lens	130 + 50
2+	Few small granules visible with low power lens	223 + 75
3+	Numerous small granules in all marrow particles	406 + 131
4+	Large granules in small clumps	727 + 248
5+	Dense large clumps of granules	1618 + 464
6+	Very large deposits obscuring marrow details	3681 + 1400

Interpretation and correlation of above data results were analyzed accordingly.

Figure1

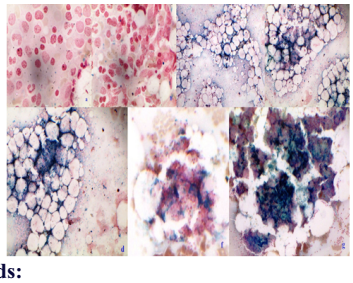

Figure legends:
Perl's stain; a) 1000x : No iron granule observed (0), b) 1000x: Small granules in reticulum cells seen only (1+), c) 100x Few small granules visible with low power lens (2+), d) 100x Numerous small granules in all marrow particles (3+), e) 100x Large granules in small clumps (4+), f) Dense large clumps of granules (5+), g) 100x Very large deposits obscuring marrow details (6+)

RESULTS

The present study was undertaken to correlate levels of serum ferritin, iron, TIBC levels with bone marrow iron morphological grading (0-VI) in 100 consecutive cases of BMA to evaluate dynamics of iron metabolism in various conditions. On the basis of clinical features, hematological findings and therapeutic trials the patients were divided into three major groups (anemias, infectious diseases and malignancies) with nine subgroups. Among all patients, 47 of anemia (male = 27, female = 20), 29 of infectious diseases (male = 27, female = 2), and 24 patients of malignancies (male = 22, female = 2) were included in the study [Table 2]. This study had more number of male patients with anemia as Command Hospital (Air Force) Bangalore is a defence service hospital.

Table 2: DETAILED DISTRIBUTION OF ALL PATIENTS AS PER DIAGNOSIS

Clinical diagnosis	Male		Female		Total	
	No	%	No	%	No	%
ANEMIAS						
Iron deficiency anemia	6	7.9	6	25.0	12	12.0
Anemia of chronic disease	9	11.8	7	29.2	16	16.0
Megaloblastic anemia	9	11.8	3	12.5	12	12.0
Sideroblastic anemia	2	2.6	3	12.5	5	5.0
Hemolytic anemia	1	1.3	1	4.2	2	2.0
INFECTIOUS DISEASES						
Acute infection	22	28.9	1	4.2	23	23.0
Chronic infection	5	6.6	1	4.2	6	6.0
MALIGNANCY						
Hematological malignancy	17	22.4	2	8.3	19	19.0
Non-hematological malignancy	5	6.6	-	0.0	5	5.0
Total	**76**	**100**	**24**	**100**	**100**	**100**

Anemia:
In IDA (bone marrow iron grade 0), 100% patients of pure IDA were diagnosed with serum ferritin <11 µg/L. In cases with co-morbidities, if the cutoff value of serum ferritin raised to 90 µg/L, then 95% cases were diagnosed. These cases with raised serum ferritin were associated with infections, inflammations and malignancies indicate its acute phase reactant nature. All IDA cases were having corresponding mean values of Hb, serum ferritin, iron and TIBC 7.281.83 gm/dl, 24.0825.72 µg/L, 67.7552.24 µg/dl, 443.4256.44 µg/dl respectively. ACD cases with BM iron grade 0 to IV had mean levels of Hb, serum ferritin, iron and TIBC, 6.28-8.65 gm/dl, 65.25-695 µg/L, 59-232 µg/dl, 267-56.44 µg/dl respectively. In megaloblastic anemia, most of the patients had adequate bone marrow iron ranging from grade I-III with mean values of serum ferritin, iron, TIBC being 219.59125.74 µg/L, 145.92141.65 µg/dl, 298.4295.68 µg/dl respectively. The two cases with BM iron grade 0 had combined nutritional deficiency anemia i.e. megaloblastic anemia and IDA. [Table 3] This study had very small number of sideroblastic anemia (5) and hemolytic anemia (2) cases for any statistical evaluation [Table 3].

Table 3: MEAN LEVEL OF STUDY PARAMETERS IN ALL CASES

Diagnosis	Hb gm/dl	S. ferritin µg/L	S. iron µg/dl	S.TIBC µg/dl
ANEMIAS				
IDA	7.281.82	24.0825.72	67.7552.24	443.4256.44
ACD	7.722.33	473.25279.13	123.44102.99	227.2567.50
Megaloblastic anemia	6.832.39	219.58125.74	145.92141.63	298.4295.68
Sideroblastic anemia	5.891.15	549.80120.99	314.60138.00	263.0057.21
Hemolytic anemia	5.000.71	392.00149.91	272.506.36	215.0025.46
All cases of anemias	6.862.34	298.49265.03	141.64126.89	303.89111.31
INFECTI. DISEASES				
Acute infection	11.712.83	532.69167.48	81.9135.79	287.26107.45
Chronic infection	8.383.27	562.00304.17	95.0038.34	82.83154.18
All infectious cases	11.023.18	538.76196.74	84.6236.03	286.34115.41
MALIGNANCY				
Hematolo. Malign.	9.274.09	416.58289.28	126.5856.57	302.53108.92

Nonhemato. Malign.	11.281.55	695.20181.87	83.6037.66	252.80124.98
All malignancies	9.693.77	474.65290.86	117.6355.39	292.17111.47
Total of all cases	**8.743.48**	**410.44273.88**	**119.3495.67**	**295.99111.68**
Significance of all cases	**F=19.097; P<0.001****	**F=9.028; P<0.001****	**F=3.341; P=0.041***	**F=0.236 P=0.790**

INFECTIONS:

Twenty nine cases of infectious diseases showed BM iron grade I-V with mean values of Hb, serum ferritin, iron and TIBC 5.60-12.70 gm/dl, 240-800 µg/L, 61-84.50 µg/dl and 145.50-439 µg/dl, 439 µg/dl respectively. All six cases of chronic infectious illnesses had BM iron grade II to V with BM iron grade II to V with corresponding ranges of Hb, serum ferritin, iron and TIBC 5.60- 9.15 gm/dl, 180-800 µg/L, 59-143 µg/dl, 145.50-454.50 µg/dl respectively [Table 3].

Malignancies:

Among patients with malignancies, most cases had BM iron grade II-IV, with serum ferritin (201-800 µg/L) along with four cases (BM iron grade 0, serum ferritin 35-135 µg/L). This indicates synthesis of ferritin by tumor cells or release from damaged cells [Table 3].

Overall interpretation:

On the basis of above findings of BM iron grading with corresponding serum ferritin (significance value F = 65.589, P < 0.001), serum iron (F=3.270, P < 0.001), TIBC (F=7.029, P < 0.001) maximum variability of bone marrow iron grade with serum iron followed by TIBC was observed [Table 4,5].

Table 4: MEAN LEVELS OF S. FERRITIN, IRON, TIBC ACCORDING TO BM IRON GRADE

BM iron grade	S. ferritin		S. iron		S. TIBC	
	Range	Mean SE	Range	Mean SE	Range	Mean SE
Grade 0	2-198	40.097.72	25-277	82.5612.58	138-518	377.4923.21
Grade I	115-365	184.2936.68	25-384	161.4346.65	295-441	369.7122.43
Grade II	116-800	408.6430.87	25-452	96.9614.89	156-475	281.6617.88
Grade III	257-800	594.2632.87	38-378	129.0016.44	159-478	270.1121.42
Grade IV	565-800	680.9127.36	52-372	201.4539.37	153-357	231.9121.53
Grade V	695-800	779.0021.00	38-386	124.0065.76	126-275	172.6059.37
Grade VI	-	-	-	-	-	-
Significance	**F=65.589; P<0.001****		**F=3.270; P<0.001****		**F=7.029; P<0.001****	

Table 5: PAIRWISE SIGNIFICANCEOF BM IRON GRADE WITHS.FERRITIN,IRON, TIBC

	Pairwise significance of S. ferritin					Pairwise significance of S. iron					Pairwise significance of S. TIBC				
	Gd I	Gd II	Gd III	Gd IV	Gd V	Gd I	Gd II	Gd III	Gd IV	Gd V	Gd I	Gd II	Gd III	Gd IV	Gd V
Gd 0	0.133	<0.001**	<0.001**	<0.001**	<0.001**	0.222	0.943	0.180	0.003**	0.866	0.999	0.004**	0.001**	0.001**	<0.001**
Gd I	-	0.002**	<0.001**	<0.001**	<0.001**	-	0.533	0.980	0.939	0.980	-	0.269	0.496	0.045*	0.010*
Gd II	-	-	<0.001**	<0.001**	<0.001**	-	-	0.631	0.018*	0.989	-	-	0.996	0.698	0.195
Gd III	-	-	-	0.454	0.057+	-	-	-	0.301	1.00	-	-	-	0.894	0.326
Gd IV	-	-	-	-	0.744	-	-	-	-	0.598	-	-	-	-	0.864
Gd V	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gd VI	-	-		-	-	-	-	-	-	-	-	-	-	-	-
	Serum ferritin values are increasing progressively as BM iron is increased.					Serum iron values are variably increasing/decreasing as BM iron is increased.					Serum TIBC values are variably increasing/decreasing as BM iron is increased.				

Stastical data proved that P value of BM iron grading is < 0.001 with both serum ferritin and TIBC, however overall correlating best with serum ferritin (Spearman correlation 0.865) as compared to TIBC and serum iron. The minus sign of TIBC indicates the inverse relationship between BM iron grade and TIBC [Table 6].

Table 6: SPEARMAN CORRELATION OF BM IRON GRADE WITH S. FERRITIN, IRON,TIBC

Pair	Spearman correlation	P value
BM iron grade vs. s. ferritin	0.865	< 0.001**
BM iron grade vs. s. iron	0.239	0.016*
BM iron grade vs. TIBC	-0.497	< 0.001**
BM iron grading is< 0.001 with both s. ferritin, TIBC, however overall correlating best with s. ferritin (Spearman correlation 0.865) as compared to TIBC, s. iron.		

It is also evident that BM iron grading correlates best with serum ferritin in all three major groups as well as in the subgroups as compared to iron and TIBC. Therefore serum ferritin can be used as reliable biochemical assay for the corresponding BM iron stores in majority of cases except those associated with infection, inflammations and malignancies [Table 7].

Table 7: PEARSON CORRELATION OF BM IRON GRADE WITH S.FERRITIN,IRON, TIBC

Diagnosis	Number of patients	BM iron vs. s. ferritin	BM iron vs. s. iron	BM iron vs. s. TIBC
Anemia	47	0.899**	0.550**	-0.572**
Infection	29	0.713**	-0.090	-0.506*
Malignancy	24	0.877**	0.94	-0.502*
Total	**100**	**0.871****	**0.290****	**-0.522****
BM iron grading is correlating best with s. ferritin in all three categories individually as well as overall, as compared to TIBC and s. iron. The minus sign of TIBC indicates the inverse relationship.				

DISCUSSION

The word 'Anemia' is derived from Greek word 'Anemia' (an=not, naime=blood) i.e. not having blood. Anemia is functionally defined as an insufficient RBCs mass to adequately deliver oxygen to peripheral tissues.[11] It is a clinicopathological condition, which is present when hemoglobin level of the body is below the normal range for the age and sex of the individual. World Health Organization defines the lower limit of normal Hemoglobin (Hb) concentration at sea level to be 12.0 gm/dl in adult women and 14.0 gm/dl in adult men11 whereas in elderly (>65 years) to be 8.5 gm/dl.[12]

Main storage compound of iron in the body are ferritin (ferric iron-apoprotein complex) and hemosiderin with former present predominantly in liver, spleen and bone marrow. Ferritin molecules (iron content 16-23%) are water-soluble and are present in plasma in equilibrium with ferritin molecules of histiocytes. Most of the non-circulating iron in the RBC's is stored in the Fe+++ and in marrow histiocytes as hemosiderin (insoluble, more stable and less available) and is available to nearby RBC's precursors. Bone marrow iron stores start decreasing earlier than clinical manifestations of anemia and lowering of hemoglobin level. Decreased iron stores are reflected by decreased serum ferritin in plasma.

Various direct and indirect methods are available to evaluate iron stores. Direct methods may be noon-invasive or invasive. Non invasive direct methods are nuclear resonant scattering of X-rays (NRS), dual energy computed tomography (DE-CT) and magnetic susceptibility. The invasive direct methods are BMA, bone marrow biopsy (BMB), liver biopsy and quantitative phlebotomy. Among these BMA (gold standard for measuring body iron stores) is preferred, since it is safer and more familiar to hematologists.

Indirect methods of measuring body iron stores are now becoming popular, being non-invasive, quantitative and more acceptable. These comprise of serum ferritin, iron, TIBC (included in the present study), transferrin saturation, free erythrocyte protoporphyrin, red cell ferritin

and urinary iron excretion with chelating agents. The first immunoassay of serum ferritin was developed by Addison et al in 1971, however there is considerable variation in results of serum ferritin levels measured by different commercial immunoassay techniques.[13]

In the present study all the twelve cases of IDA had BM iron grade 0, out of them eight of pure IDA (ferritin < than 12 µg/L in IDA) correlate with Miller et al and Lopez et al. Raised serum ferritin (41-80 µg/L) of four cases of mixed types (IDA + infections) were similar to Ahluwalia et al, WHO17 (ferritin 6-185 µg/L) and Nadeem.[14-18] The mean value of TIBC (443.42+56.44 µg/dl) was found to be high in all cases of IDA. There were directly proportional and indirectly proportional relationship of BM iron grade with serum ferritin and TIBC respectively, therefore these two parameters can be used to distinguish between IDA and ACD cases.[3,19] Serum iron levels showed more variation with corresponding BM iron grades.

Twelve cases among sixteen of chronic inflammatory diseases had BM iron grade II-IV (ferritin 301-800 µg/L) in accordance with Bableshwar et al (ferritin 40-1134 µg/L).[20] Birgegard G. et al 1980 concluded that serum ferritin during infection is of glycosylated type, whereas intracellular ferritin is not glycosylated, suggesting its elevation during infection is caused by release along the normal pathways, i.e. an augmented synthesis, not by leakage.

Among twelve cases of megaloblastic anemia, ten cases had adequate BM iron grade of I-III with corresponding s. ferritin 115-435 µg/L in accordance with Hussein et al. The increased iron stores in these cases are due to ineffective erythropoiesis. The other two cases with diminished BM iron grade were in accordance with Lipschitz indicating the presence of mixed anemia i.e. megaloblastic and iron deficiency anemia.[21]

Although the number of sideroblastic anemia (five) and hemolytic anemia (two) were too small for any statistical analysis, the findings of raised serum ferritin and increased bone marrow iron stores were in accordance with Camaschella et al and Mishra AK et al.[22]

Twenty nine cases of infectious diseases had BM iron grade I-IV with corresponding s. ferritin 101-800 µg/L were corresponding with KDOQI, Gangat et al, Cullis et al and Bari MA et al.[23-26]
Nineteen cases of hematological malignancies had BM iron grade I-IV with corresponding serum ferritin 101-800 µg/L, similar to the findings of Lee EJ et al and Eyetsemitan et al.[27] Value of low hemoglobin in these cases were corresponding to Naoum et al.[28] The high values of serum ferritin in all malignancies could be due to its release by damaged cells or associated hepatic dysfunction impairing clearance or direct synthesis from the tumor cells.

CONCLUSION

In the index study serum ferritin, iron and TIBC values were correlated with bone marrow iron grading (0-VI) in 100 consecutive cases of BMA to evaluate dynamics of iron metabolism in various diseases.

It can be finally concluded that serum ferritin levels are a reliable indicator of bone marrow iron stores in majority of cases except those associated with infections, inflammations or malignancies. Therefore its estimation will reduce the invasive technique of BMA to access the iron stores. Further the measurement of serum ferritin levels is particularly useful in detecting early iron deficiency and to differentiate between IDA and ACD.

ONFLICT OF INTEREST: None to declare
FUNDING: No funding was obtained

COMPLIANCE WITH ETHICAL STANDARDS:

Ethical standards have been well maintained and followed as per existing institutional guidelines. A written consent was taken from patients before the bone marrow aspiration procedures.

This article does not contain any studies with animals performed by any of the authors.

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