Serum ferritin as a biomarker in hematological conditions

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Abstract

Objective: This study is to evaluate significance of serum ferritin in differentiating between different hematological conditions.
Methods: This is a prospective study conducted on children aged between 1 to 12 years attending Pediatric OP and IP of Navodaya Medical College, Raichur for period of one year. 200 children who fulfilled WHO criteria for anemia were taken as cases and 200 normal healthy children of same demographic data were taken as controls. Serum ferritin levels were compared between the normal healthy children and children with different types of anemias.
Results: The mean value of serum ferritin in normal controls (106.8 ± 38.0ng/ml), in IDA (5.1 ± 2.3ng/ml), in Dimorphic anemia (330±40ng/ml), in Sickle cell anemia (430±83ng/ml), in Thalassemia major (1520±230 ng/ml), and in leukemias (1550±140ng/ml)
Conclusion: Serum ferritin is moderately raised in dimorphic anemia, in Sickle cell anemia, Thalassemia and leukemias it is significantly raised. And we found that serum ferritin is the most sensitive and reliable parameter compared to other parameters in iron profile in evaluating both iron deficient and iron overload conditions.

Keywords: Serum Ferritin, Thalassemia, Sickle cell anemia, Dimorphic anemia, leukemia.

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Introduction

Ferritin is the storage protein of Iron and is found in blood, liver, spleen, bone marrow and intestine (mucosal cells). Iron bound to ferritin is non-toxic, as the free iron is toxic and catalyzes the conversion of O^{-}_{2} to hydroxyl $OH^{\cdot}$ Oxy radicals[1]. Ferritin reflects true iron stores and is not susceptible to the short-term variations that occur with serum iron levels and TIBC. Since ferritin is an acute phase reactant it can be elevated with liver disease, malignancy, and chronic renal disease. Iron deficiency anemia is likely if the ferritin level is less than 15 ng per mL (15 mcg per L) in an otherwise healthy child.[2]

Iron stores in the body exist primarily in the form of ferritin. The ferritin molecule composed of an apoprotein called Apoferritin, with a molecular weight of 550,000. It is composed of 24 monomeric units, each having molecular weight of 18,000, that form a spherical shell. There are six pores in the shell that have catalytic activity, most notably the binding of ferrous iron (Fe^{++}) and its subsequent oxidation to "ferric oxy hydroxide" (FeO.OH). Bound form of iron with ferritin is more soluble and iron is present as "ferric oxyhydroxy phosphate" complex in ferritin. Upto 4500 Fe^{+++} atoms are found stored in a ferritin complex.[1] Normally, there is a little ferritin in human plasma. However, in patients with excess iron, the amount of ferritin in plasma is markedly elevated. A low serum ferritin value reflects depleted iron stores, but not necessarily the severity of the depletion as it progresses.[3] The amount of ferritin in plasma can be conveniently measured by a sensitive and specific radioimmunoassay and serves as an index of body iron stores.

Normal concentrations of Ferritin vary by sex and age. Concentrations are high at birth, rise during the first two months of life, and then fall throughout later infancy[4]. At about one year of age, concentrations begin to rise again and continue to increase into adulthood; males have higher values than females. Values among men peak between 30–39 years of age and then tend to remain constant until about 70 years of age.

Serum ferritin has shown a greater sensitivity and predictive value in subjects with uncomplicated anemia and without anaemia.[4,5] The estimation of serum ferritin not only being a confirmatory test, but also have a specific characteristic that is able to identify the risk subjects before they become symptomatic. Serum ferritin estimation is the most accurate test that strongly indicates the iron status of an individual within normal range as well as iron deficiency and excess. It is very important to diagnose the iron deficiency in young growing children who has got a very weak balance between iron stores, requirement and supply before it results in hypochromic microcytic anaemia.[6]

The clinical role of the serum ferritin assay lies in its tendency to parallel the size of iron stores during normal development as well as in iron deficiency and iron overload. The serum ferritin assay seems to have

an important advantage over serum iron and iron-binding capacity in that low values are almost invariably diagnostic.[7]

Significant rise in serum ferritin in thalassemia major patients might be due to repeated blood transfusions, ineffective erythropoiesis, increased gastrointestinal iron absorption and ineffective management of iron overload by iron chelators in some patients. In inflammation, infection and malignancy serum ferritin acts as acute phase reactant thus its elevation is seen in these conditions.[8] Due to blockage in reutilization of reticuloendothelial storage iron (ferritin), in inflammatory and other conditions there is a low level of haemoglobin, decreased serum iron, decreased transferrin saturation and high level of serum and tissue ferritin.[9]

The objective of the present study was to delineate the problem of clinical anemia, with low / normal / high serum ferritin levels and the use serum ferritin levels along with other hematological parameters in approach to type of Anemia.

Materials and Methods

The present prospective study was conducted on children attending Pediatric OP and IP of Navodaya Medical College, Raichur for period of one year with an estimated sampling size of 200 cases and 200 controls. The present study was carried out with the prior approval from the Institutional Ethics Committee.

Inclusion criteria:

- Age group: 1 to 12 years of age
- Anemic children clinically diagnosed (as per WHO criteria for anemia), attending pediatric OP and IP of Navodaya Medical College, Raichur.

Exclusion criteria:

- Age group: Above 12 years of age and below 1 year.
- Anemia due to acute blood loss.
- Those who refused consent.
- Patients outside Raichur district of Karnataka.

Sampling method:

- Age based Random Sampling.
- Children attending pediatric OPD and Inpatients at Navodaya Medical College, Raichur.

Collection of data

After obtaining a written consent, 3ml blood was withdrawn aseptically from large peripheral veins of all selected children is transferred into a plain tube and centrifuged at 3000rpm for 10 min to separate serum. Serum ferritin concentration was determined by using Vidas Ferritin an automated quantitative test using Enzyme Linked Fluorescent Assay technique. The fluorescence level was measured by automated analyzer (Biomeraux mini Vidas). Whereas, Serum Iron and TIBC were estimated by Biosystems Iron and TIBC kit in semiautoanalyzer, (Erba Chem 5plus) which uses Ferrozine method. Transferrin saturation was calculated as Serum iron × 100 / TIBC

Statistical Analysis

Values were calculated as mean±SD and the statistical analysis was done using SPSS 17.0 software. Student's unpaired t-test was used to study serum iron, TIBC transferring satutation % and serum ferritin levels in iron deficiency subjects. The p-value of <0.05 was considered as statistically significant.

Results

Table 1: Gender distribution of Cases and Controls

Sex	Cases		Controls		Total
	Number	Percentage	Number	Percentage	
Males	92	46%	92	46%	184
Females	108	54%	108	54%	216
Total	200	100	200	100	400

In the current study, Males are (46%) and Females were (54%) in both the cases and controls. (Table 1).

Table 2: Socio-economic status of the family of cases and controls

	Cases		Controls	
SES	Number	Percentage	Number	Percentage
Class I-III	48	24%	158	**79%**
Class IV-V	152	76%	42	21%
Total	200	100%	200	100%

In the present study, according to modified Kuppu Swamy classification, our findings showed that Anemia is 76% in class IV/V and 24% in class I/II/III respectively (Table 2).

Table 3: Distribution of anaemias based on etiology

Etiology	Number	Percentage
Iron deficiency anaemia	80	40%
Megaloblastic anaemia	38	19%
Thalassemia major	30	15%
Thalassemia trait	19	9.5%
Sickle cell anemia	12	6.0%
Anemia of chronic disease and inflammation	12	6.0%
Anemia of CKD	09	4.5%
Total	200	100%

In this study, Iron deficiency anemia (40%) was the most common type followed by Megaloblastic anemia (19%) and Thalassemia major (15%). Thalassemia trait (9.5%), sickle cell anemia (12%), anemia of chronic disease and inflammation (6.0%), and with least common type being anemia of CKD (Table 3).

Table 4: Study of serum ferritin levels in cases and controls

Serum ferritin levels	Cases (200)		Controls (200)		Total
	No	Percent	No	Percent	
0- 15µg/L	110	55	00	0	110
16- 30 µg/L	12	06	34	17	46
31-150 µg/L	06	03	158	79	164
151-500 µg/L	52	26	08	04	60
>500 µg/L	20	10	00	00	20

In the present study the serum ferritin levels in 96% of normal subjects fall between 15-150 µg/L, 55% cases between 0-15 µg/L, 06% cases between 16-30 µg/L, 03% cases between 31-150 µg/L, 26% cases between 151-500 µg/L and 10% >500 µg/L (Table 4).

Table 5: Mean values for parameters of Iron profile in Cases with Iron deficiency anemia

Groups	Hb%	Serum Iron(µg/dl)	TIBC(µg/dl)	Serum Ferritin(ng/ml)	Transferrin Sat%
Controls	12.3±1.5	76.5±32.5	322.4±32.9	136.8±34.0	24±10.6
Cases	5.3±1.2	28.6±10.3	496.0±99.5	5.1±2.3	6.3±2.7

From the present study normal values of iron profile are derived mean values of different parameters being serum Iron (µg/dl) was 76.5±32.5, TIBC (µg/dl) 322.4±32.9, Serum Ferritin (ng/ml) 106.8±38.0, Transferrin Sat% 24±10.6. In the present study mean values of different parameters in IDA were derived Serum Iron(µg/dl) 28.6±10.3, TIBC(µg/dl) 496.0±99.5, Serum Ferritin(ng/ml) 5.1±2.3, Transferrin Sat% 6.3±2.7. Except TIBC all other parameters of iron profile are significantly lower in IDA as compared with controls (Table 5).

Table 6: Data Showing Significance of Serum Ferritin as Index of Iron Stores Compared with Other Tests

Tests Performed	Patients	Total Cases	Specificity (%)	Sensitivity (%)
TIBC	Iron Deficient	80	15 (18.75%)	65 (81.25%)
	Total Anemic	200	56 (28%)	144(72%)
Transferrin Saturation	Iron Deficient	80	4(5%)	66 (95%)
	Total Anemic	200	32(16%)	168 (84%)
Serum Ferritin	Iron Deficient	80	0 (0%)	80(100%)
	Total Anemic	200	30(15%)	170 (85%)

In present study serum ferritin was more precise and sensitive with values <15ng/ml in all IDA subjects with 100% sensitivity as compared to other parameters in iron profile. Second most specific parameter in this study was transferrin, which was less than 16 percent in 95 percent of iron deficient anemic cases and above 16 percent in 5 percent cases. In anemic children the sensitivity of serum ferritin was 85% and specificity 15%. It was less than the value of 15 ng/ml in all iron deficient children. It is 100% sensitive compared to other parameters in iron profile (Table 6).

Table 7: Comparison of mean values of serum ferritin levels in different hematological conditions with that of controls

Etiology	Serum ferritin(ng/ml)	p-value
Iron deficiency anemia	5.1 ± 2.3	<0.001
Dimorphic anemia	330±40	<0.05
Thalassemia major	1520±230	<0.001
Sickle cell anemia	430±83	<0.05
Leukemias	1550±140	<0.001
Controls	106.8 ± 38.0	

In the present study mean of serum ferritin levels in patients with dimorphic anemia is 330±40ng/ml, which is significantly higher than that of normal subjects. In the present study mean value of serum ferritin in thalassemia major patients is 1520±230ng/ml, which was significantly elevated than normal subjects. In the present study mean value of serum ferritin in sickle cell anemia patients is 430±83ng/ml, which was significantly elevated than normal subjects. In the present study mean value of serum ferritin in leukemia patients is 1550±140ng/ml, which was significantly elevated than normal subjects (Table 7).

Discussion

The clinical role of the serum ferritin assay lies in its tendency to parallel the size of iron stores during normal development as well as in iron deficiency and iron overload. The concentration of iron storage compounds and of serum ferritin are both high at birth, increase further for 1-2 months, and then decrease rapidly, maintaining low levels after 6 months of age. Diminution of iron stores during this period is a normal developmental consequence of rapid growth during a period when the major physiologic source of food, i.e., milk, is a poor source of iron.[10]

Although serum ferritin concentration seems to reflect the size of iron stores under most conditions, there may be certain exceptions; infections and acute leukemia are two examples. Increased serum ferritin concentrations have also been reported in adults with acute myeloblastic leukemia, chronic leukemia, Hodgkin's disease, and in children with acute lymphoblastic leukemia.[11] The typical Indian diet is based on cereals and pulses which contain more than 40% of total phosphorus as phytates and vegetables and plant food contain oxalates, which interfere with absorption of food iron inspite of high dietary intake.[12]

In this study mean value of serum ferritin in 200 normal healthy children 106.8±38.0 ng/ml. These values are in accordance with the observations made by earlier studies[13-14]. Our results have shown that 96% of serum ferritin values in normal children fall in between 15-150 ng/ml. This value correlates with the studies done by earlier authors[15-18]

In the present study serum ferritin level is very low in IDA with mean value of 5.1±2.3 ng/ml, similar observation where also observed in earlier studies[13-14]. In present study 38 children are having Megaloblastic anemia whose mean serum ferritin was significantly higher than mean of normal healthy controls. This corresponds with the study done by Hussein and co-workers[19]. In the present study among 30 patients with Thalassemia major mean serum ferritin levels were so high. This corresponds with the studies done by Nadeem and co-workers[20], whereas in study conducted by Martti and co-workers[8], on 7 Thalassemia major subjects the range of serum ferritin level is between 590-1800 ng/ml which is also very high and correlates with present study. This significant rise in serum ferritin in thalassemia major patients might be due to repeated blood transfusions, ineffective erythropoiesis, increased gastrointestinal iron absorption and ineffective management of iron overload by iron chelators in some patients.

In the present study among 19 patients of thalassemia trait serum ferritin levels are within normal range. This corresponds to the studies done by Sonay and co-workers.[21] In the present study among 12 patients of sickle cell anemia, the mean serum ferritin was significantly greater than controls which corresponds to the studies done by Hussain and co-workers[22].

Injured hepatocytes leak ferritin into the serum this might be the reason for elevation of serum ferritin in present study. In inflammation, infection and malignancy serum ferritin acts as acute phase reactant thus its elevation is seen in these conditions. In inflammatory and other conditions there is blockage to reutilization of reticuloendothelial storage iron (ferritin), thus there is a low level of hemoglobin, decreased serum iron, decreased transferrin saturation and high level of serum and tissue ferritin.

To conclude, Serum ferritin is moderately raised in dimorphic anemia, in Sickle cell anemia, Thalassemia and leukemias it is significantly raised. And we found that serum ferritin is the most sensitive and reliable parameter compared to other parameters in iron profile in evaluating both iron deficient and iron overload conditions.

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