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Association of Serum Ferritin and Hematological Parameters in Investigation of Iron Deficiency Anemia

Jamil Akhtar[1], Waheed Alam[2], Fatima Khan[2], Jaleel Kamran[3]

[1] *College of Medical Laboratory Technology, National Institute of Health, Islamabad*
[2] *Public Health Laboratories Division, National Institute of Health, Islamabad*
[3] *Watim Dental College Rawalpindi*

Correspondence: *Jamil Akhtar*
jamilakhtarcmlt@gmail.com

Abstract

Objective: To find out a correlation between serum ferritin and hematological parameters and age of patients visiting National Institute of Health, and also to establish the role of this biochemical indicator in the diagnosis of IDA.

Methodology: This comparative cross-sectional study was conducted in Endocrinology and Special Chemistry Department of PHLD, National Institute of Health Islamabad from July 2019 to December 2020. A total of 235 patients including males and females of different age groups, manifesting anemic clinical history, diminished hematological parameters and showing microcytic hypochromic blood film, were selected for the study. Sysmex K-1000 auto analyzer and Roche Elecsys 2010 immunoassay analyzer were used for the measurements of various hematological parameters and ferritin respectively. Stained blood films were also prepared to examine red blood cell morphology and their staining pattern.

Results: Serum ferritin results were statistically analyzed against Hb and other hematological parameters. Significant correlation ($P > 0.05$) was observed in low serum ferritin groups with iron deficiency anemia of both males and females. However, this correlation was not observed in normal and high ferritin groups of both genders. Also low serum ferritin levels did not show any strong correlation with the age of the patients. Similarly, it was also found that when serum ferritin significantly varies from subnormal values towards normal or above normal levels, a significant difference results in Hb levels accordingly and more or less in other hematological parameters, indicating a strong interdependence. No such relationship in serum ferritin and age of the patient was observed. The results were also supported by hypochromic, microcytic blood picture and by investigating clinical history of the patient.

Conclusion: Iron deficiency anemia can be treated and cured with proper diagnosis, diet and treatment. Therefore, to investigate and differentiate iron deficiency anemia among other different types of anemias, serum ferritin determination is decisive in diagnosis as it decreases only in this type of anemia as is established in the study conducted.

Key words. Ferritin, Hematological parameters, Iron deficiency, Anemia

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Introduction

Iron deficiency anemia (IDA) is one of the most common types of anemia whose major indicator is a reduced concentration of hemoglobin (Hb) in microcytic red blood cells, as there is an insufficient supply of iron. Ferritin is an iron storing intracellular protein and is required for the release of iron in a controlled manner hence acting as a buffer against iron deficiency and iron overload in the body.[2]

Iron deficiency (ID) is one of the most common nutritional deficiencies and is the leading cause of anemia in children, elderly and adult women. Early recognition of ID is crucial to prevent systemic complications of the disease. Such early diagnosis, by

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necessity relies on laboratory testing.[1]

Iron deficiency anemia (IDA) is the most common type of anemia met within clinical practice. It is a major public health issue in developing as well as developed countries.[2] It occurs at all stages but is especially common in women of child bearing age in whom the main etiological factors are menstruation, pregnancy, pathological blood loss and deficient diet. In adult males and postmenopausal women pathological blood loss and in infants and children, deficient diet and diminished iron stores at the birth are the main factors causing IDA.[3]

In laboratory investigations decreased concentration of hemoglobin is an essential feature. The total red blood cell count (TRBC), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) are usually reduced depending on severity of anemia. In the blood film the red cells are hypochromic and microcytic. Thus IDA is also classically termed as microcytic anemia[4, 5]. Iron is absorbed in the body and does not exist as free iron, rather is stored in ferritin protein. Ferritin and transferrin act as cargo for iron and release it in a controlled fashion.[6, 7]

Ferritin can act as a suitable marker to check for iron deficiency. Amongst biochemical finding, a serum ferritin level of 12 ng/ml or less is diagnostic of ID[8,9]. Ferritin levels are reduced in ID but usually remain normal or increased in other hypochromic anemias. The diagnosis of IDA is based on the determination of biochemical indicators of iron status like serum ferritin, clinical features such as history and physical examination of the patient, hematological indicators like Hb and red cells indices and characteristic morphological features of RBCs stained blood film which are microcytosis and hypochromia.[3]

The determination of serum ferritin is a suitable method for ascertaining the iron metabolism situation. If the depressed level is accompanied by hypochromic microcytic blood picture it manifests presence of IDA.[10, 11] Anemia is a reduction in the number of red blood cells in the amount of Hb (iron containing pigment of RBCs that carries oxygen from lungs to tissues), another related index called hematocrit (the volume of RBCs after they have been spun in a centrifuge). In IDA, RBCs are abnormally small (microcytes) and pale (hypochromic) when a blood film slide is microscopically examined.[15] To calculate the prevalence of IDA and to characterize iron status of persons a multiple indicator model including ferritin, Hb estimation can be employed[12] as in United States.

The serum ferritin is a universally available and well standardized measurement that has been the single most important laboratory measure of iron.[13, 14] Phlebotomy studies in normal subject have demonstrated that one ug/litre serum ferritin correspond to 8 to 10 mg or 120 ug storage iron per kg body weight. For over a decade research has been available to show that it is the best and most reliable indicator of iron status, decisive in iron IDA when shows reduced levels.[15] Thus the aim of present study was to find out association of serum ferritin and hematological parameters for investigation of iron deficiency anemia among persons presenting anemic history and hypochromic microcytic blood picture.

Materials and Methods

235 Patients of different age groups and both genders, clinically suspected of anemia, referred from different hospitals and clinics of Rawalpindi and Islamabad to National Institute of Health Islamabad were selected for evaluation of serum ferritin levels for research study during the year 2019 and 2020.

A proforma, designed for the purpose, was filled with particulars of the patient and their physical history. Registration and documentation was also processed at reception of Public Health Laboratories Division. Strictly following standard operative procedures (SOPs) of phlebotomy and blood collection procedures, 5cc of venous blood was collected by venipuncture technique. The same was distributed equally into two tubes. First half in a clean dry, sterile centrifuge tube to obtain serum for ferritin estimation and the next half collected in an anticoagulant (EDTA 1.5 mg/ml of blood) tube for determination of hematological parameters and for morphological studies of RBCs.

Serum ferritin levels were determined in blood samples of patients by using the Roche Elecsys 2010 Immunoassay Analyzer. It calculates the ferritin concentration of each sample in ng/ml or ug/l. Measuring range for the instrument is 0.5 – 2000 ng/ml.

Investigations for hematological parameters were performed on sysmex K-1000 auto analyzer using EDTA anticoagulated blood. Hb levels on all blood

samples were determined. Anemia is present when the Hb level in the blood is below the lower extreme of the normal range for the age and sex of the individual. Red blood cell counts were also performed and this parameter is an alternative means of assessment of anemia. MCV, MCH, and MCHC were also estimated, these are equally helpful in the investigation of type of anemia. These have low values in Iron deficiency anemia.

For morphological examination of RBCs, stained slides of thin blood films were made on whole blood samples, fixed and stained with Leishman's stain and examined microscopically to estimate increased variations in size and shape of RBCs referred as anisocytosis and poikilocytosis respectively. Abnormal sizes known as microcytosis and acrosytosis and variation in staining intensity known as hypochromia or hyperchromia were noted to interpret the results. In iron deficiency anemia microcytic hypochromic picture is seen. Normal diameter of RBC is 6.7 to 7.7. um with average 7.2 um. If this value is decreased RBCs are microcytes. Similarly RBCs show normally central paler area after staining that is one third of the diameter. If central paler area is increased cells are called hypochromic.

At the end all the numerical data was entered in Microsoft Excel, sorted out, grouped and statistically analyzed to find out significant correlation of serum ferritin with other blood parameters and to interpret results accordingly.

Results

In male patients' correlation between serum ferritin < 30 ng/ml (that is below normal) and hematological parameters was determined (Table-1). Values in boldface indicated a significant correlation ($P<0.05$). Ferritin was strongly correlated with Hb, HCT, MCV, MCH and MCHC. Hb showed the highest number of significant correlations with other parameters.

In a similar way in female patients' correlation between ferritin <13ng/ml (that is below normal) and hematological indicators was determined (Table-2). Again values in boldface indicated a significant correlation ($P<0.05$). Ferritin showed significant correlation with Hb and other hematological parameters. Hb again showed highest number of correlation when compared to other parameters.

Next when correlation between ferritin with normal levels (Male 30-400 ng/ml, females13-150 ng/ml) and hematological parameters was determined (Table 3 to 4). Results showed that in both sexes ferritin level did not have significant correlation with other parameters.

Lastly, correlation of ferritin levels > 400 ng/ml and > 150 ng/ml (values above normal) in males and females was determined with hematological parameters (Table 5,6). Similarly, ferritin did not show strong correlation with Hb and other hematological parameters.

Table 1: Correlation between ferritin and the studied blood complete picture (CP) parameters.

	Ferritin	TRBC	Hb	HCT	MCV	MCH	MCHC	Age
Ferritin	-							
TRBC	0.2736	-						
Hb	0.6461	0.5035	-					
HCT	0.5827	0.7006	0.9494	-				
MCV	0.4904	-0.0255	0.8170	0.6865	-			
MCH	0.5090	-0.0341	0.8310	0.6525	0.9677	-		
MCHC	0.5198	0.0626	0.8344	0.6316	0.8420	0.9407	-	
Age	0.2552	0.0370	0.4338	0.4419	0.5756	0.4868	0.3534	-

Values in the boldface indicate a significant correlation ($p<0.05$) Group: males with serum ferritin< 30 ng/ml (below normal).

Table 2: Correlation between ferritin and the studied blood complete picture (CP) parameters.

	Ferritin	TRBC	Hb	HCT	MCV	MCH	MCHC	Age
Ferritin	-							
TRBC	-0.1133	-						
Hb	**0.3927**	**0.3504**	-					
HCT	**0.3367**	**0.5239**	**0.9449**	-				
MCV	**0.4734**	**-0.3927**	**0.6816**	**0.5578**	-			
MCH	**0.4287**	**-0.3118**	**0.7420**	**0.5555**	**0.9268**	-		
MCHC	**0.1943**	0.0979	**0.4832**	**0.3094**	**0.4535**	**0.5657**	-	
Age	-0.0445	0.0073	0.1407	**0.1818**	0.1483	0.0936	-0.1142	-

Values in the boldface indicate a significant correlation ($p<0.05$)
Group: females with serum ferritin< 13 ng/ml (below normal).

Table 3: Correlation between ferritin and the studied blood complete picture (CP) parameters.

	Ferritin	TRBC	Hb	HCT	MCV	MCH	MCHC	Age
Ferritin	-							
TRBC	-0.2555	-						
Hb	-0.0484	0.7493	-					
HCT	-0.1177	0.8351	0.9795	-				
MCV	0.1549	-0.5503	0.0436	-0.0707	-			
MCH	0.2185	-0.5620	0.0180	-0.1156	0.9142	-		
MCHC	0.3752	-0.3078	0.2179	0.0412	0.5112	0.7227	-	
Age	-0.1220	0.1335	0.4308	0.4188	0.3080	0.2363	0-1377	-

Values in the boldface indicate a significant correlation (p<0.05) Group: males with serum ferritin 30-400 ng/ml (normal).

Table 4: Correlation between ferritin and the studied blood complete picture (CP) parameters.

	Ferritin	TRBC	Hb	HCT	MCV	MCH	MCHC	Age
Ferritin	-							
TRBC	0.0782	-						
Hb	0.1971	-0.0148	-					
HCT	0.1602	0.0667	0.9567	-				
MCV	0.1290	-0.5922	0.6608	0.5880	-			
MCH	0.1522	-0.5480	0.7321	0.6082	0.9708	-		
MCHC	0.2243	-0.1549	0.6690	0.4374	0.5294	0.7047	-	
Age	0.3565	-0.0321	0.5425	0.5320	0.3803	0.4222	0.3852	-

Values in the boldface indicate a significant correlation (p<0.05)
Group: females with serum ferritin 13-150 ng/ml (normal).

Table 5: Correlation between ferritin and the studied blood complete picture (CP) parameters.

	Ferritin	TRBC	Hb	HCT	MCV	MCH	MCHC	Age
Ferritin	-							
TRBC	-0.6929	-						
Hb	-0.6408	0.9422	-					
HCT	-0.6522	0.9501	0.9970	-				
MCV	-0.1058	0.0427	0.3520	0.3399	-			
MCH	-0.2086	0.2406	0.5417	0.5157	0.9526	-		
MCHC	-0.4622	0.5832	0.6982	0.6473	0.3214	0.5797	-	
Age	-0.5235	0.4478	0.5513	0.5811	0.5329	0.5268	0.2805	-

Values in the boldface indicate a significant correlation (p<0.05) Group: males with serum ferritin>400 ng/ml (above normal).

Table 6: Correlation between ferritin and the studied blood complete picture (CP) parameters.

	Ferritin	TRBC	Hb	HCT	MCV	MCH	MCHC	Age
Ferritin	-							
TRBC	-0.2715	-						
Hb	-0.0417	**0.6989**	-					
HCT	-0.2075	**0.8650**	**0.9250**	-				
MCV	0.1534	-0.5027	0.2168	-0.0066	-			
MCH	0.3144	-0.4946	0.2722	-0.0564	**0.9178**	-		
MCHC	0.4070	-0.2823	0.3584	-0.0210	0.5747	**0.8487**	-	
Age	0.4764	-0.2085	0.0009	-0.0494	0.3172	0.2867	0.1892	-

Values in the boldface indicate a significant correlation (p<0.05)
Group: females with serum ferritin>150 ng/ml (above normal).

Discussion

Anemia is present when the hemoglobin level in the blood is below the lower extreme of the normal range for the age and sex of the individual. The hemoglobin level is employed as the prime arbiter in the diagnosis of anemia. Among different types of anemias an important class is hypochromic microcytic anemias which include iron deficiency anemia, anemia of chronic disease, thalassemia and sideroblastic anemia. In clinical practice iron deficiency anemia needs to be differentiated as it is the most common micronutrient deficiency anemia in the world.[3]

Serum ferritin is the most clinically useful and applicable test for the diagnosis of iron deficiency anemia as it reflects total body iron stores.[24] The most commonly used thresholds of serum ferritin used in

different earlier studies are SF<12,SF<15, SF<20 AND SF<30(ng.ml^{-1}). However a SF<30(ng.ml^{-1}) has been used in a number of studies because its sensitivity has been found 90%. [22]

The present study was aimed to investigate iron deficiency anemia among patients exhibiting anemic history and hypochromic microcytic blood picture by establishing an association and correlation between serum ferritin levels and hematological indicators. In the study different groups of both males and females were made on the basis of serum ferritin level as SF< normal range; SF> normal range and SF within normal range. For all these groups data was statistically analyzed and correlation between serum ferritin and blood CP parameters and also with the age of the patient was determined keeping in view the normal ranges of different parameters under consideration.

Results suggest that for normal and higher ferritin level groups of both males and females, no significant correlation exist between ferritin and hematological indicators ($p>0.05$) i.e. hemoglobin, TRBC, HCT, MCH &MCHC and thus it excludes iron deficiency anemia. It is in agreement with an earlier study carried by Guyatt.[16]

On the other hand ferritin levels less than the lower limit of the normal range group of both males and females exhibit strong correlation with Hb, HCT, MCV, MCH and MCHC and results are indicative of iron deficiency anemia. These results are in agreement with earlier studies performed by Karimi, Halis & Pongstaporn.[2, 17, 18]

Thus ferritin is a strong biochemical indicator for investigating iron deficiency anemia. Its low levels combined with diminished values of Hb and other hematological indicators along with hypochromic microcytic blood picture and anemic history differentiate IDA from other clinical types of anemias.[21, 22, 23]

Conclusion

Serum ferritin has strong association with hematological parameter in investigation of iron deficiency anemia. Its low level i.e. less than the normal reference ranges in both genders along with decreased values of Hb, HCT, MCV, MCH and MCHC give a diagnosis of iron deficiency anemia. The scheme of this panel of testing can effectively be used for screening IDA in population of individuals at high risk.

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