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Original Research Article

Study of Serum Ferritin to Diagnose Iron Deficiency Anaemia in Moderately to Severely Anaemic Patients in a Tertiary Health Care Setting

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Abstract

Introduction: One out of every third person is suffering from anaemia throughout the world; moreover half of this anaemic population are suffering with iron deficiency. The credibility of serum ferritin and transferrin for the diagnosis of Iron Deficiency Anaemia (IDA) is still suspicious as different studies have shown different results. *Material and Methods:* This was a cross sectional study conducted in the pathology department of a Tertiary Health Care Setting. Study population consisted anaemic patients of 18 to 60 years of both sexes. Bone marrow was aspirated and smears were stained using the May-Grunwald-Giemsa stain. With an automated hematology analyzer blood counts were measured. Serum transferrin receptor assays were performed using a commercially available kit based on a polyclonal antibody in a EIA format. *Results:* The present study included 100 patients suffering from anaemia. Out of these 100 patients, 42 anaemic patients were having iron deficiency; while 58 patients were having non iron deficiency etiology. Transferrin saturation level was significantly low in 11.63% in IDA patients compared to 30.2% in anaemic patients without iron deficiency with *p*-value <0.01. Serum ferritin level was significantly lower in IDA patients (78.6 ± 33.9 ng/mL vs 180.8 ± 46.2 ng/mL, $p<0.01$) in comparison to patients without iron deficiency etiology of anaemia. *Conclusion*: Finding of current study showed that high values of Serum transferrin receptor assays and low level of serum ferritin and transferrin saturation levels has been found associated with IDA. These results suggest that transferrin and serum ferritin should be considered as reliable markers for the diagnosis of IDA. Moreover, this method may be used as a lesser-invasive technique for the diagnosis of IDA; instead of using invasive and painful methods like bone marrow aspiration test. However, studies on larger populations are required to establish serum ferritin and transferrin studies as primary determinants for the diagnosis of IDA.

Keywords: IDA; Anaemia; Serum ferritin; Transferrin.

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Introduction

One out every third person is suffering from anaemia throughout the world; moreover half of this anaemic population are suffering with iron deficiency.[1] Anaemia and iron deficiency have been found as associated factors for various other disorders and medical conditions like cognitive and cardio-vascular disorders, osteopenia, muscle weakness etc for human beings.[2] It has been found that prevalence of anaemia increases with the advancement of age; moreover, various co-morbidities like nutritional disorders, chronic renal failure and gastrointestinal disorders have been found associated with anaemia especially iron deficiency anaemia.[3–6]

The credibility of serum ferritin and transferrin studies as the primary diagnostic modalities of iron deficiency anaemia (IDA) is still not validated as different studies have shown different results. Serum ferritin level from 40 to 200 ng/mL is considered as a well known marker of iron store in few studies.[7–9] Bone marrow aspiration is one of the best diagnostic tools for verifying iron deficiency in patients suffering with IDA. However, despite more accuracy bone marrow aspiration is an invasive and painful technique. Recent studies suggest that various factors influence serum ferritin without altering iron stores especially in patients suffering with inflammatory disorders. Nevertheless, serum ferritin level is one of the important adjunct diagnostic method for the diagnosis of IDA.[10,11] There is still a dilemma about the efficacy of transferrin studies and serum ferritin levels for the diagnosis of IDA as few studies have shown that transferrin studies and serum ferritin are not among the excellent markers for the measurement of iron stores.[12]

Therefore, present study was designed to determine the efficacy of transferrin studies and serum ferritin in predicting the iron store of patients suffering from iron deficiency anaemia. Moreover, purpose of current study was to assess the diagnostic properties of Serum Ferritin at different levels for the diagnosis of IDA.

Materials and Methods

This was a cross sectional study conducted in the pathology department of a Tertiary Health Care Setting. Present study included hundred anaemic patients selected randomly which were registered in our tertiary care hospital. Study population consisted of anaemic patients of 18 to 60 years, of both sexes. Study population was divided into two groups; Group I consisted IDA patients while Group II included Anaemic patients without IDA. The informed written consent was taken from each and every patient before they participated in the study. All patients underwent a bone marrow examination to define the type of anaemia and to ascertain iron stores. The subjects with haemoglobin level lower than 13 g/dL and 12 g/dL in men and in women respectively were classified as having anaemia and are included in the study.

Exclusion criteria

Subjects those taking iron supplements, and with a history of blood transfusion within one year before the start of the study, haematological malignancies, chronic renal failure and on haemodialysis, were excluded.

Bone marrow was aspirated and smears were stained using the May-Grunwald-Giemsa stain. With an automated hematology analyzer, blood counts were measured. Serum transferrin receptor assays were performed using a commercially available kit.[13] The central 95th percentile of the reference distribution of TfR concentration is 0.85 to 3.05 mg/L. [13] The reference ranges of Serum ferritin for men and for women is 15 to 306 mg/L and 5 to 103 mg/L respectively. Transferrin reference range was 2.1 to 3.4 mg/L for men and 2.0 to 3.1 mg/L for women. Reference range of Serum Iron was 10 to 40 mmol/L in both sexes.[13]

Statistical Analysis

Results of the present study compared the transferrin studies and serum ferritin of both groups. Youden's index was used to record optimal cut-off levels that differentiated patients of both groups at the highest sensitivity and specificity. All the variable distributions were tested for normality using the Kolmogorov–Smirnov test. The *p*-value less than 0.05 was considered as statistically significant. The Statistical software SPSS V18 manufacture by USA was used for the entire calculations.

Results

The present study included 100 patients suffering with anaemia. Out of these 100 patients, 42 patients were suffering with IDA; while 58 patients were non-IDA patients. Results showed that there was an insignificant difference between age of patients with IDA (58.8 ± 18.4 yrs) and patients without IDA

(59.4 ± 16.5 yrs) with *p*-value >0.05. There was a significant difference between haemoglobin levels of patients with IDA (9.4 ± 2.7 g/dL) and patients without IDA (10.1 ± 2.4 g/dL) with *p*-value <0.01. Further, serum iron was significantly low (34.6 ± 9.3 μg/dL vs 93.7 ± 16.6 μg/dL) in IDA patients compare to patients without iron deficiency anaemia. Serum Transferrin levels were significantly high in IDA patients in comparison to patients without IDA. Further, results showed transferrin saturation level was significantly low (11.63%) in IDA patients compared to anaemic patients without iron deficiency (30.2%) with *p*-value <0.01 (Table 1).

Table 1: Comparison of iron studies parameters in anaemic patients with and without iron deficiency anaemia.

Variants	IDA	Non-IDA	*p*-value
Age (Years)	58.8 ± 18.4	59.4 ± 16.5	>0.05
Haemoglobin (g/dL)	9.4 ± 2.7	10.1 ± 2.4	<0.01
Serum iron (μg/dL)	34.6 ± 9.3	93.7 ± 16.6	<0.01
Transferrin (μg/dL)	330.8 ± 21.9	258.6 ± 25.9	<0.01
Transferrin saturation level (mean,%)	11.63 ± 3.4	30.2 ± 7.5	<0.01
Serum ferritin (ng/mL)	78.6 ± 33.9	180.8 ± 46.2	<0.01

It is evident from (Fig. 1) that serum ferritin level was significantly lower in IDA patients (78.6 ± 33.9 ng/mL vs 180.8 ± 46.2 ng/mL, *p*<0.01) in comparison of patients without iron deficiency anaemia.

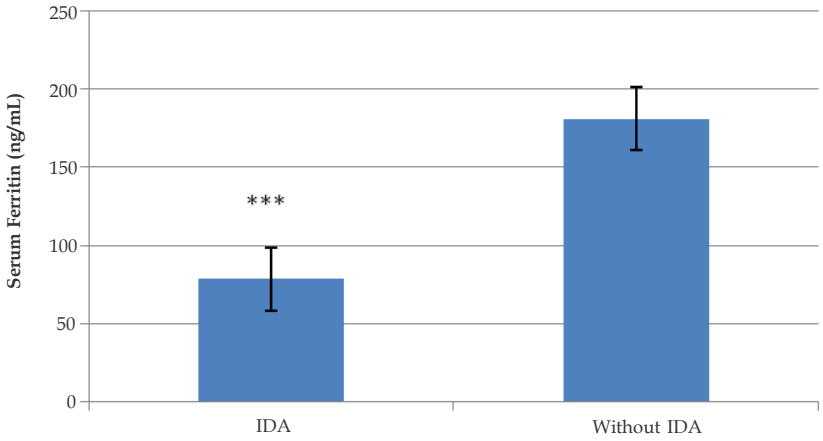

Fig 1: Comparison of serum ferritin in both groups.

Positive and negative analytical values of 44% and 75% correspondingly indicate the cut off level. In addition diagnostic performances for various other levels of SF were determined by performing different analysis. SF levels lower than 18 ng/mL showed the positive liklihood ratio of 4.9 (95% CI: 2.8–6.4), sensitivity of 17% and specificity of 94.2% for the diagnosis of IDA. Serum cut off levels of 45 ng/mL and 60 ng/mL yielded positive likelihood ratios of 2.82 (95% CI: 1.7–3.8) and 2.5 (95% CI: 1.5–2.9), respectively. In patients with SF levels less than 100 ng/mL, the likelihood of IDA decreased to 1.8 (95% CI:1.2–2.3) with negative predictive value of 75% and negative likelihood ratio of 0.67 (Table 2).

Table 2: Diagnostic properties for different cut off levels of serum ferritin in diagnosis of iron deficiency anaemia

Variants	18 ng/mL	45 ng/mL	60 ng/mL	100 ng/mL
Sensitivity (95% CI)	17 (9–22)	32 (23–36)	36 (37–46)	50 (40–64)
Specificity (95% CI)	94.2 (88–98)	85 (78–93)	81 (76–93)	72(58–80)
PPV (95% CI)	66 (44–86)	56 (41–71)	53.8 (40–67)	44.3 (34–55)
NPV (95% CI)	68.6 (62–72)	72 (63–77)	74.4 (64–80)	75 (65–84)
LR+ (95% CI)	4.9 (2.8–6.4)	2.82 (1.7–3.8)	2.5 (1.5–2.9)	1.8 (1.2–2.3)
LR− (95% CI)	0.92 (0.77–0.94)	0.83 (0.72–0.87)	0.72 (0.69–0.83)	0.67 (0.62–0.81)

Discussion

It has been difficult to diagnose the cause of decreased haemoglobin in anaemic patients. Various studies have been done to understand the direct or indirect relation of transferrin studies and serum ferritin levels with IDA.[14–16]

Results of the present study showed that haemoglobin was significantly low in IDA anaemia patients in comparison of anaemia patients without IDA. On the other hand serum iron level was significantly low in IDA patients compare to anaemic patients without Iron Deficiency. These findings are consistent with the earlier studies of Ganti AK *et al*.[17] and Chio CW *et al*.[18] as they recorded significantly low haemoglobin and serum iron level in IDA patients compare to anaemic patients suffering with other types of anaemia.

Further, results of the current study showed that Serum transferrin receptor assay levels were significantly high in IDA patients compared to patients without IDA. In contrast to this transferrin saturation was significantly low in IDA patients in comparison to patients without IDA. These findings are in agreement with earlier studies of Klantar-Zadeh *et al*.[19] and Rimon E *et al*.[20] as they observed a significantly high level of Serum transferrin receptor assay levels whereas, significantly low level of transferrin saturation levels in IDA patients compared to other types of anaemic patients. However, Klantar-Zadeh *et al*.[19] showed high sensitivity and specificity of transferrin saturation test in the diagnosis of IDA even in patients with co-morbidities.

These findings support the utility of transferrin studies for the diagnosis of IDA as various studies showed a significant relation between IDA and transferrin level.[8,20] Moreover, these studies found a negative correlation between haemoglobin level and serum transferrin level in IDA patients.[8,20]

On the other hand, significant diagnostic ability, according to the cut-off levels, for IDA has been found associated with serum ferritin.[10,14] Valuable information is provided by different range of interpretations associated with likelihood ratios which are associated expression of various diagnostic values of serum ferritin. It is considered that greater the likelihood ratios, magnitude of change will be higher from pretest to post-test probability of the target disorder.[16,21]

Present study has recorded highest sensitivity for IDA at the cut off point of 100 ng/mL. Further results showed that 50% of the patients having transferrin saturation <14%, belonged to group of patients with serum ferritin level <100 ng/mL. In addition, a higher negative predictive value in comparison to positive predictive value were recorded in 75% patients without IDA having serum ferritin level >100 ng/mL. Furthermore, our study observed diagnostic properties for different cut-off levels of serum ferritin for the diagnosis of IDA. Current study recorded that increased cut off levels of serum ferritin level was associated with higher sensitivity and higher negative predictive value. Whereas, lower cut off value of serum ferritin leads to decreased sensitivity and increased positive predictive value.

These findings are consistent with the results of previous studies of Halberg L *et al*.,[7] Milman N *et al*.[22] and Kis A *et al*.[23] as they showed that cut off points for Serum Ferritin in IDA patients vary from 12 to 100 ng/mL.

Halberg L *et al*.[7] suggested that Serum Ferritin <16 ng/mL is the best cut off level for the differentiation of IDA patients with non IDA patients with a sensitivity of 75% and specificity of 98%; Further, they recorded that serum iron store disappear as soon as Serum Ferritin levels reach up to 40 ng/mL. Milman N *et al*.[22] reported Serum Ferritin value was 100 ng/mL in men and 78 ng/mL in women for the diagnosis of IDA. Further, Kis A *et al*.[23] observed in their study that sensitivity of 64.9%, Serum Ferritin level of less than 100 ng/mL and specificity of 96.1% had high diagnostic values for the detection of IDA. Similarly, Coenen JL *et al*.[24] observed in their study that serum ferritin levels of <70 ng/ml was an accurate indicator of IDA in all the patients.

In a randomly selected sample of 38-year-old women, Serum Ferritin <16 ng/mL was the best cut off level to differentiate patients with and without iron deficiency with a sensitivity of 75% and specificity of 98%; the iron stores began to disappear at Serum Ferritin levels from 25 to 40 ng/mL.[7] Serum ferritin level is considered among the best diagnostic methods for the differentiation between IDA patients and anaemic patients without Iron Deficiency.

A lot of variation was recorded in diagnositic capability of Serum Ferritin for IDA due to various diagnostic criteria in different studies. However, studies did not establish serum ferritin as a prominent diagnostic tool for IDA unanimously.[6,7,22–26]

Serum ferritin has emerged as an effective non invasive technique for the diagnosis of IDA. However, precaution should be taken in IDA patients suffering with various co- morbidities as studies suggested that cut off value of serum ferritin for diagnosis of IDA may be higher in patients suffering with different types of inflammatory diseases.[22–24]

Conclusion

Findings of current study showed that high levels of Serum transferrin receptor assays and low level of serum ferritin has been found associated with IDA. These results suggest that transferrin studies and serum ferritin should be considered as reliable markers for the diagnosis of IDA. Moreover, this method may be used as a lesser-invasive technique for the diagnosis of IDA; instead of using invasive and painful methods like bone marrow aspiration. However, studies on larger populations are required to establish Serum Ferritin as the sole determinant for the diagnosis of IDA.

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