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## Evaluation of serum ferritin in screening for iron deficiency in tuberculosis

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**Abstract** Serum ferritin (SF) values  $\leq 10$   $\mu\text{g/l}$  are diagnostic of absent Bone Marrow Iron (BMI) stores and therefore of iron deficiency (ID). However, SF, which may be elevated as a part of acute phase reaction, is an unreliable indicator of BMI stores in the setting of chronic disorders, making it difficult to diagnose ID in these patients. Thus, in chronic disorders (CD) such as tuberculosis, bone marrow examination is the only reliable way to establish ID. This study was done in order to identify levels of SF that would be indicative of absent BMI stores and also to study a combination of hematological and biochemical parameters that would be helpful in raising the predictive power of SF in patients of tuberculosis. Fifty-five tuberculosis patients were studied and classified into Iron Deplete (ID) and Iron Replete (IR) based on BMI. Raising the cut-off values of SF from  $\leq 10$   $\mu\text{g/l}$  to  $\leq 30$   $\mu\text{g/l}$  diagnosed 88% of ID cases correctly, as compared with 61% when cut-off levels of  $\leq 10$   $\mu\text{g/l}$  were used. At cut-off values higher than 30  $\mu\text{g/l}$ , the sensitivity was markedly reduced. Therefore, raising cut-off levels of SF to  $\leq 30$   $\mu\text{g/l}$  was most effective in predicting absent BMI, especially in a population where ID is highly prevalent. Combination of SF  $\leq 30$   $\mu\text{g/l}$  with mean corpuscular volume (MCV), erythrocyte sedimentation rate (ESR) and total iron binding capacity (TIBC) did not improve the predictive power of SF further. Also, 89.5% cases could be correctly classified by logistic

regression equations using SF with ESR and C-reactive protein (CRP).

**Keywords** Iron deficiency · Anemia of chronic disorders · Serum ferritin · Tuberculosis

**Abbreviations** SF: Serum ferritin · BMI: Bone marrow iron · ID: Iron deplete · IR: Iron replete · CD: Chronic disorders · MCV: Mean corpuscular volume · TIBC: Total iron binding capacity · ESR: Erythrocyte sedimentation rate · ACD: Anemia of chronic disorders · IDA: Iron deficiency anemia · Hb: Hemoglobin concentration · TLC: Total leukocyte count · RBC: Red blood cell · RDW: Red cell distribution width · % TS: Percent transferrin saturation · SI: Serum iron

### Evaluation of serum ferritin levels in screening for iron deficiency in tuberculosis

Iron deficiency (ID) is the most common cause of nutritional deficiency anemia in the developing world [8]. There is also a high incidence of various chronic illnesses such as tuberculosis in this population. The incidence of tuberculosis is as high as 1/1000 in our population [16]. It is important to establish the presence of ID in these patients of tuberculosis and other chronic inflammatory or infectious disease, as even mild iron deficiency causes a significant impairment in the immunological status and reduces the capacity of such patients to control infections. This is of special importance in developing countries because iron deficiency, if established in these patients, could be corrected with cheap iron supplementation that would not only improve anemia but also influence the clinical outcome of the infectious disease [7].

Determination of conventional hematological indices and biochemical variables is of little help in demonstrating iron deficiency in these patients as they are similarly affected in both ID and anemia of chronic disorders (ACD) [13, 14]. Bone marrow examination for iron is the

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gold standard for detecting ID [13, 14] in such conditions. Being an invasive procedure, it causes patient discomfort and anxiety. Hence, serum ferritin (SF), a non-invasive parameter reflecting iron stores, is being extensively studied. SF  $\leq 10 \mu\text{g/l}$  is diagnostic of absent bone marrow iron stores in any clinical setting [13]. However, SF is an acute phase reactant and is increased in inflammations and infections. In most cases of CD, SF is disproportionately increased relative to iron stores [10, 12]. In such a clinical setting of CD it is, therefore, not reflective of bone marrow iron (BMI). To compensate for this inflammatory component, many authors have suggested higher cut-off values, predictive of ID in patients with anemia of chronic disorders [1, 5, 6, 8, 11, 19]. Some others have used a combination of other laboratory parameters to increase the predictive power of SF and have achieved variable results [4, 6, 8, 12, 15, 20]. This study was done in order to evaluate predictive power of SF in detecting ID in patients of tuberculosis with anemia, singly as well as in combination with other laboratory parameters, and to suggest cut-off levels with high sensitivity and specificity to detect ID with reasonable accuracy. BMI examination was taken as a gold standard to establish ID in such patients.

## Materials and methods

Fifty-five patients with tuberculosis attending a large tertiary health care provider in Delhi were selected for the study. Anemia was diagnosed using WHO recommendation of Hb  $< 13 \text{ g/dl}$  (males) and  $< 12 \text{ g/dl}$  (females) and only patients with microcytic hypochromic anemia (MCV  $< 80 \text{ fl}$ ) who had iron deficiency anemia as a likely possibility were included. Patients with other causes of anemia and those on hematinic therapy were excluded. All patients were informed about the nature of the study and an informed consent was taken. A complete Hemogram: hemoglobin concentration (Hb), total leucocyte concentration (TLC), red blood cell (RBC) count, red cell distribution width (RDW) (done on Sysmex K-1000), ESR (westergrens method), CRP (Rhelax CRP slide agglutination test), peripheral smear examination (Wright's stain), reticulocyte count [2] (assessed by two independent observers), Serum Iron (SI) [9], total iron binding capacity (TIBC) [17], percent transferrin saturation (%TS) and SF (Diagnostic Automation, USA, Microwell ELISA) were done in all patients. Red cell ferritin assay was performed by preparing a hemolysate of the packed cells after removing the buffy coat and then measuring its ferritin content [3].

As BMI is a gold standard in detecting ID, all the patients in the study were subjected to bone marrow aspiration. Smears were stained with Pearl's stain [2] and assessed and graded using a scale of 0–6+ [13] by two independent observers.

In the next stage of the study, subjects were divided in two groups, Iron Deplete (ID) and Iron Replete (IR), on the basis of bone marrow iron staining. Patients with 0+ iron were categorized as ID while those with 1+ and more iron as IR. Various hematological and biochemical parameters were compared between the two groups for any statistically significant difference. Parameters that were found significant were then analyzed further for their efficacy in predicting BMI. As SF represents iron stores of the body and was significantly different in the two groups, its predictive power was studied at cut-off values of  $\leq 10, 20, 30, 50, 70$  and  $90 \mu\text{g/l}$  using chi-square analysis. In our study SF  $\leq 30 \mu\text{g/l}$  was identified as most representative of absent BMI. Hence, combination of SF  $\leq 30 \mu\text{g/l}$  with MCV  $< 76 \text{ fl}$ , TIBC  $> 400 \mu\text{g/dl}$ , ESR  $> 50 \text{ mm/1}^{\text{st}} \text{ h}$ , CRP positivity and RBC ferritin  $\leq 10 \text{ ag/RBC}$  (other parameters which were significantly different in the two groups) were tested, using chi-square analysis, for their contribution in improving the predictive power of SF. Also, regression equations were prepared for assessing predictive power of ferritin alone and in combination with other inflammatory parameters (ESR, CRP) to predict the bone marrow iron stores.

## Results

The 55 adult patients under study (31 females, 24 males) had various manifestations of tuberculosis (35.1%– Pott's spine; 26.3% tuberculosis of hip; 38.6% miscellaneous) and duration of illness ranged from 6 months to 2 years. There were 39 ID and 16 IR cases on BMI examination. Table 1 shows the various hematological and iron parameters in the two groups. MCV, ESR, TIBC and SF were significantly different in the two groups. RDW and TS, which are considered to be useful parameters in discriminating ID deficiency from other causes of microcytic hypochromic anemia, were not significantly different in our study. On further analysis of the frequency distribution of MCV (Fig. 1), it was seen that a large number of ID patients (36.4%) had very low MCV of  $< 74 \text{ fl}$  as compared with the IR group, where patients with very low MCV were few (12.4%). This signified that severe degree of microcytosis was of a greater relevance when ID coexisted with CD than without. An interesting observation was made while studying the frequency

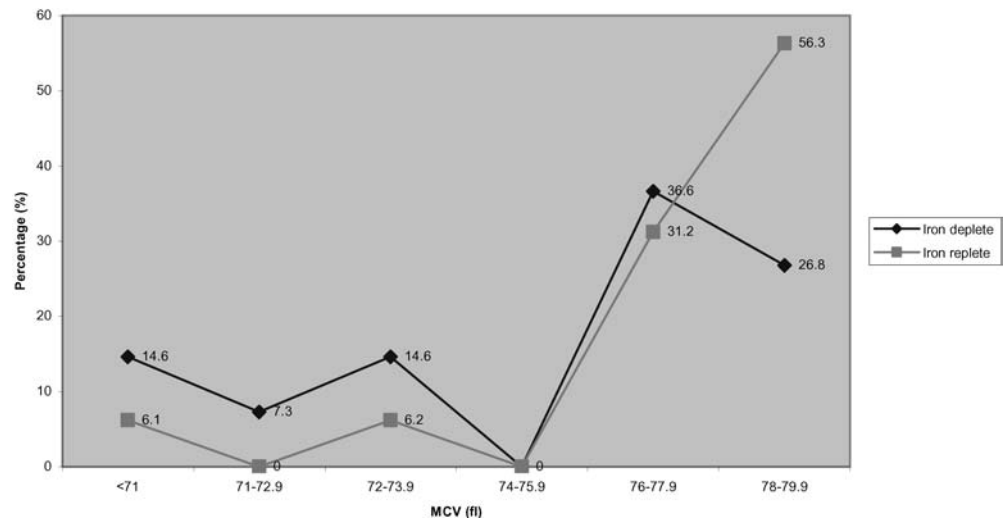
**Table 1** Mean and ranges of hematological and iron parameters in ID and IR patients

Parameters	Total (55)		ID (39)		IR (16)		P-value
	Mean	Range	Mean	Range	Mean	Range	
Hb, g/dl	9.6 $\pm$ 1.8	5–12	9.6 $\pm$ 1.7	5–11	9.7 $\pm$ 2.1	5–12	0.887
MCV, fl	76 $\pm$ 3.7	66–80	75.6 $\pm$ 4	66–80	77.8 $\pm$ 2.4	70–79	0.021*
RDW, %	15.9 $\pm$ 3.2	11.4–24.7	16.3 $\pm$ 3.2	11.4–23.9	15.1 $\pm$ 3.1	11.4–24.7	0.191
ESR, mm/1st h	55 $\pm$ 2.04	25–105	49.4 $\pm$ 20.7	25–105	63.8 $\pm$ 25.1	35–105	0.003*
SI, $\mu\text{g/dl}$	53 $\pm$ 18.8	16–107	54.4 $\pm$ 20.1	16–107	47.9 $\pm$ 14.6	31–60	0.241
TIBC, $\mu\text{g/dl}$	353 $\pm$ 71.1	216–527	370.2 $\pm$ 64.6	240–527	309.6 $\pm$ 70.2	216–449	0.003*
TS, %	14.9 $\pm$ 10.1	4.2–50.9	17.1 $\pm$ 10.1	4.2–20.7	19.8 $\pm$ 10.0	10.8–48.9	0.367
SF, $\mu\text{g/l}$	35 $\pm$ 46.9	$< 10$ –230	17.7 $\pm$ 16.7	$< 10$ –100	91.9 $\pm$ 104.1	20–230	0.012*
RBC Ferritin, ag/RBC	13.1 $\pm$ 14.7	0.2–64	11.7 $\pm$ 14.7	0.2–64	16.9 $\pm$ 14.28	0.2–53.3	0.81

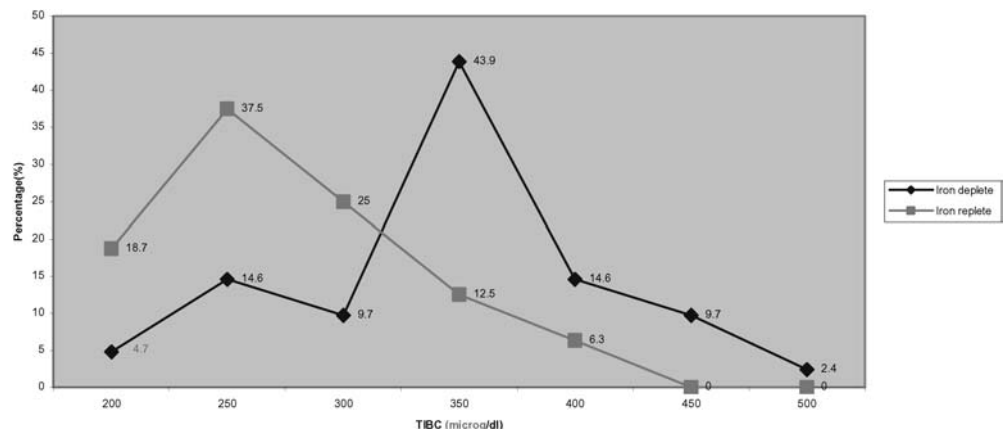
Figures in parentheses denote number of patients

\* Denotes significant value

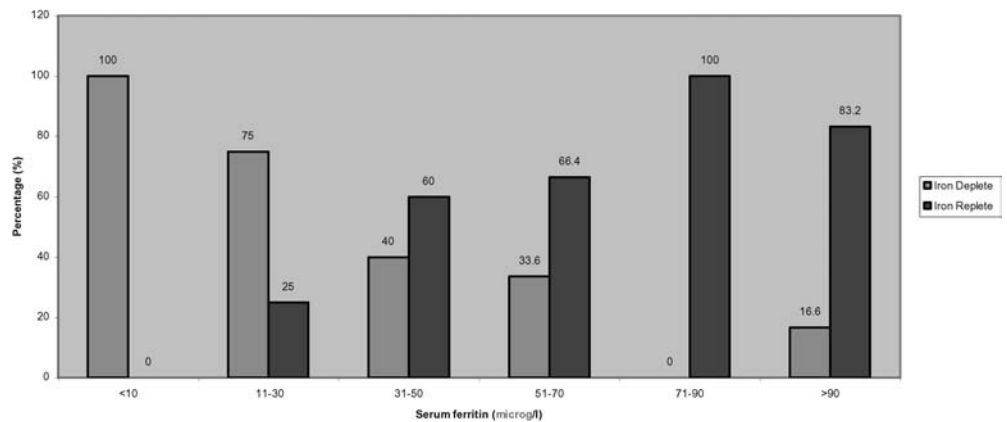
**Fig. 1** Frequency distribution curve of MCV in ID and IR patients



**Fig. 2** Frequency distribution curve of TIBC in ID and IR patients



**Fig. 3** Relative distribution of SF in ID and IR patients at different cut-off levels of SF



distribution of TIBC (Fig. 2). IR patients had low values of TIBC. There was only one patient in the IR group who had TIBC >400  $\mu\text{g}/\text{dl}$ . Our study did not show any significant difference in RDW and RBC ferritin in the two groups. Although RDW was higher in patients of ID group, the difference was not significant ( $p < 0.191$ ) and therefore precluded its use for discriminating between the two groups.

The correlation between SF and BMI was significant ( $p < 0.013$ ). Further, the relative distribution of SF at various ranges in the two groups was studied (Fig. 3). It was observed that there was no patient in IR group with SF  $\leq 10 \mu\text{g}/\text{l}$ . All the patients at this cut-off were ID. Thereafter, relative percentage of patients in ID group decreased with increasing levels of SF. There was no ID case in which SF was in the range of >70–90  $\mu\text{g}/\text{l}$ . Only

**Table 2** Predictive power of serum ferritin in combination with MCV, TIBC, ESR, CRP and RBC ferritin

Parameters	Sensitivity, %	Specificity, %	Positive predictive power, %	Negative predictive power, %
A	92.3	65.9	29.8	93.8
B	100	28.1	31.7	100
C	62.5	26.5	12.2	81.3
D	80	29.8	19.5	87.5
E	92.8	48.2	63.4	87.5

A SF <30 µg/l and MCV <76 fl, B SF <30 µg/l and TIBC>400 µg/dl, C SF <30 µg/l and ESR >50 mm/1st h, D SF <30 µg/l and CRP positivity, E SF <30 µg/l and RBC ferritin <10 ag/RBC

one case with high SF level (>90 µg/l) was seen in ID group. This patient was an 18-year-old female who had high fever at the time of assessment and had an ESR of 60 mm/1st h. These results encouraged us to find a suitable cut-off value at which most of the ID cases could be identified without subjecting a patient to a bone marrow examination. Chi-square analysis was performed at various cut-off levels of SF, ranging from ≤10 to ≤90 µg/l. Predictably, values ≤10 µg/l were effectively able to define ID and values >70 µg/l were able to exclude ID. At SF levels of ≤10 µg/l, only 23/39 patients (58%) were classified as ID. However, at values ≤30 µg/l, 36 out of 39 patients (92%) were correctly classified as ID. Cut-off value of ≤30 µg/l was considered best as it had maximum sensitivity (90%) as well as highest negative predictive power (75%) with a reasonable specificity (75%) and positive predictive power (90%). It was observed that the sensitivity of SF decreased with higher cut-off values, whereas when lower cut-off values were used, specificity decreased dramatically.

#### SF in combination with other laboratory parameters

Chi-square analysis was done using SF ≤30 µg/l with other parameters (Table 2) to assess the improvement, if any, in the predictive power of SF ≤30 µg/l. Combination of SF ≤30 µg/l with MCV and TIBC was slightly more sensitive (MCV 92.3%; TIBC 100%) than SF used alone (90%), but had markedly low specificity (MCV 65.9%; TIBC 28.1%). Therefore, they did not offer any added advantage. No other parameter ( ESR<CRP and RBC Ferritin) was noteworthy of improving the predictive power of SF.

#### SF in combination with inflammatory parameters

SF is an acute phase reactant and its rise parallels that of other inflammatory parameters. Its combination with inflammatory parameters was tested in order to correct the acute phase component of SF so that it could correctly predict BMI. Logistic regression analysis was done for SF with inflammatory parameters, namely SF with ESR; SF with CRP; SF with ESR and CRP.

The logistic equation used was:  $P = 1 / 1 + e^{-Z}$ , where  $P$  = probability of predicting BMI.  $Z$  is a variable that

depends on the value of the parameters used for assessing BMI.

$$Z = 0.04 \times \text{ESR} + 0.063 \times \text{SF} - 5.256; \quad (1)$$

when ESR and SF used.

$$Z = 0.084 \times \text{SF} - 0.084 \times \text{CRP} - 3.311; \quad (2)$$

when CRP and SF used.

$$Z = 0.1340 \times \text{SF} - 0.174 \times \text{CRP} + 0.082 \times \text{ESR} - 9.193; \quad (3)$$

when ESR, CRP and SF are used.

If  $P < 0.5$ , then the case belonged to ID group. If  $P > 0.5$ , then the case belonged to IR group.

Using these equations, ESR, CRP and SF were able to predict BMI with a very high degree of accuracy. A combination of SF and CRP could correctly identify 84.2% of cases correctly, and a combination of SF and ESR had a slightly better accuracy in predicting BMI (89.5%), whereas combination of SF, ESR and CRP did not further increase the predictive power of SF when used in combination with ESR alone (89.5%). Hence, equations using logistic regression of SF with inflammatory parameters were best in predicting BMI; however, cut-off values of SF ≤30 µg/l also gave reasonably accurate prediction.

## Discussion

Detection of iron deficiency in anemia of chronic disorders has always been a subject of predicament. This is especially important in populations where prevalence of ID is high. Assessment of bone marrow iron stores is considered the gold standard in detecting iron deficiency in such a setting [13]. SF, a non-invasive indicator of BMI is an acute phase reactant and hence increases as a part of acute phase response in inflammations and infections [10]. Therefore, its value in predicting absent BMI stores at cut-off levels of ≤10 µg/l becomes unreliable in CD such as tuberculosis in our study. To overcome this problem, some authors have used cut-off values ≤60 µg/l for SF as diagnostic of ID [11], while others have suggested cut-off values ≤70–90 µg/l [1,5,6,8,19]. No consensus has been reached with respect to the ideal cut-off value of SF that could predict BMI reliably as few authors have attempted a correlation of SF with BMI

using BMI as the gold standard for establishing iron deficiency. In this study of 55 patients, SF  $\leq 30$   $\mu\text{g/l}$  provided a reasonable level of sensitivity (90%) and positive predictive power (90%) and fairly good specificity (75%) and negative predictive power (75%).

To further raise the predictive power of SF at cut-off levels of  $\leq 30$   $\mu\text{g/l}$ , a combination of SF with other parameters was studied. Witte et al., in their study of 43 cases, suggested that ferritin concentration, if corrected for acute phase response, could estimate BMI even in inflammatory conditions [20]. However, Kuerer et al. could not demonstrate an increase in predictive capacity of SF using combination with other inflammatory parameters [12]. Beck and associates proposed a multivariate approach to estimate iron stores. They proposed that, because readily available tests were partial predictors of bone marrow iron stores, a properly weighted combination of tests could be helpful [4]. In our study, using a similar approach we were able to further raise the predictive power of SF when combined with ESR and CRP. Using these inflammatory parameters, we developed logistic regression equations that were able to detect as high as 89.5% of cases correctly. However, simple combination of SF  $\leq 30$   $\mu\text{g/l}$  along with ESR and CRP positivity was of no help in increasing the predictive power of SF.

MCV and TIBC also showed a significant difference between the two groups and were further studied at various cut-off values to assess their ability to increase the predictive power of SF at  $\leq 30$   $\mu\text{g/l}$ . SF  $\leq 30$   $\mu\text{g/l}$  in combination with MCV  $< 76$  fl had a very good sensitivity (92.3%) but had very low specificity (28.1%) and positive predictive power (31.7%). Similarly, combination of SF  $\leq 30$   $\mu\text{g/l}$  with TIBC  $> 400$   $\mu\text{g/dl}$  had a sensitivity and negative predictive power of 100% but had very low specificity (65.9%) and positive predictive power (29.8%).

Recently, the role of RBC ferritin in reflecting BMI has been studied. Balaban et al. found that RBC ferritin as a single test is more sensitive than SF per se in predicting ID. However, they too found that SF had a better correlation with BMI as compared to RBC ferritin [3]. In this study, using cut-off value of SF  $\leq 30$   $\mu\text{g/l}$  and RBC ferritin  $< 4$  ag/RBC, we found the combination to be more specific and sensitive than SF or RBC ferritin when used alone. In our study, the combination had a sensitivity of 92.8% and a negative predictive value of 87.5% but specificity was extremely poor (48.2%). Besides, estimation of RBC ferritin is a cumbersome procedure.

Therefore, we suggest that in population groups with a high prevalence of ID, a lower cut-off value of  $\leq 30$   $\mu\text{g/l}$  is reflective of absent BMI stores as compared with higher cut-off values suggested by authors in the west. At higher levels, although the specificity gradually improved, an increasing number of ID cases were missed; therefore, higher cut-off values as suggested by earlier authors were not helpful in screening for ID in cases of tuberculosis (a good screening test should have a high sensitivity and positive predictive power). To conclude, SF level of  $\leq 30$   $\mu\text{g/l}$ , which is higher than the usual cut-off of

$\leq 10$   $\mu\text{g/l}$  used in uncomplicated ID, is recommended to reasonably diagnose ID in a setting of tuberculosis and other CD. Combination of SF with inflammatory parameters had very good efficiency in detecting ID, provided equations derived from logistic regression were used. These equations, though apparently cumbersome (made simple by use of scientific calculators or computers), could be of value in decision making in individual cases.

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