DIAGNOSTIC ACCURACY OF SERUM FERRITIN AND SOLUBLE SERUM TRANSFERRIN RECEPTOR, TAKING BONE MARROW IRON STAIN AS A GOLD STANDARD FOR IRON DEFICIENCY ANEMIA IN HETEROGENOUS GROUP OF PATIENTS

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ABSTRACT

Objective: To determine the diagnostic accuracy of serum ferritin and soluble serum transferrin receptor (sTfR), taking bone marrow iron stain as a gold standard for iron deficiency anaemia in heterogeneous group of patients.

Study Design: Cross-sectional diagnostic accuracy study.

Place and Duration of Study: Department of Diagnostic, Combined Military Hospital Lahore, from Mar to Aug 2020.

Methodology: A total of 55 adult patients, of both genders, undergoing bone marrow examination for any reason were enrolled. Patients with known hemolytic condition (sickle cell anemia, megaloblastic anemia), taking erythropoietin/iron supplements, transfused red cell concentrate (RCC) recently or undergoing chemotherapy were excluded. Age, gender, clinical history and results of bone marrow examination, complete blood count (CBC), serum Ferritin and C-reactive protein (CRP) were recorded.

Results: Serum ferritin was found to be less sensitive (28%) but more specific (100%) for reflecting reduced bone marrow iron stores as compared to sTfR (sensitivity: 60%, specificity: 96.6%). sTfR had highest likelihood ratio (15) and diagnostic accuracy (80%). On Receiver Operator Characteristic (ROC) graph Transferrin index (AUC=0.908) showed maximum accuracy, followed by Ferritin (AUC=0.884) and sTfR (AUC=0.879).

Conclusion: Serum soluble transferring receptor (sTfR) and transferrin index has advantage over serum ferritin alone in predicting the bone marrow iron stores and differentiating iron deficiency anemia from anemia of chronic disease.

Keywords: Anemia of chronic disease (ACD), C-reactive protein (CRP), Ferritin, Iron deficiency anemia (IDA), Serum soluble transferring receptor (sTfR).

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INTRODUCTION

Iron deficiency anemia (IDA) and Anemia of chronic disease (ACD) are common types of anemia in clinical practice.[1] Since long, serum ferritin has been used as an indicator of body iron stores. Serum Ferritin is highly specific but not a sensitive marker of iron deficiency. Being an acute phase reactant, Ferritin is falsely raised in patients with acute and chronic illnesses, masking iron deficiency.[2] In the patients with co-morbids, it becomes difficult to discriminate between these two types of anemias i.e. IDA and ACD.

The accurate diagnosis of iron deficiency is essential for prompt diagnosis and successful management as it may be the sign of serious illness like GIT malignancy.[3] There is a need of better marker of body iron stores, with increased sensitivity and specificity. In this context, serum soluble transferrin receptor (sTfR) was evaluated. sTfR is a truncated monomer of tissue receptor which mediate cellular uptake of iron, which is released in plasma in transferrin cycle of iron uptake.[4] It's a marker of erythropoietc activity thus less specific for iron deficiency. Erythroblast and reticulocytes are the main source of sTfR.[5] Iron deficient cell expresses more sTfR and raised sTfR levels are seen in iron deficiency. Unlike ferritin, sTfR is not an acute phase reactant; hence it is not affected by acute or chronic illness, making it a sensitive marker of iron deficiency.[6] Thus reducing the need of unnecessary bone marrow examination.

Transferrin index is calculated by dividing the sTfR with log of serum ferritin.[7] The confounding factors which affect serum Ferritin and sTfR, does not affect its ratio, i.e. sTfRs/log Ferritin and it shows better correlation with bone marrow iron stores. In our study we compared serum ferritin, sTfR levels and transferrin index, which amongst them reflect the bone marrow iron stores better in heterogeneous group of patients.

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METHODOLOGY

This cross-sectional diagnostic accuracy study was carried out at Diagnostic Department of Combined Military Hospital Lahore, from March to August 2020, after taking approval from Research Review Board of the hospital (IRB-certificate No: 161/2020). The sample size was calculated from the following equation (A1) taken from a study by Flahault *et al*, on Sample size calculation in diagnostic test studies.[8]

$$\frac{[z_{1-\beta}\sqrt{\pi(1-\pi)}+ z_{1-\alpha}\sqrt{(\pi-\delta)(1-\pi+\delta)}}{\delta^2}$$

(A1) 55 patients were recruited using non-probability convenient sampling. Informed consent was obtained from them.

Inclusion Criteria: Patients undergoing bone marrow examination for the first time for any reason like PUO, unexplained anemia, bicytopenia, weight loss or suspected malignancy were included in the study.

Exclusion Criteria: We excluded the patients with sickle cell anemia, megaloblastic anemia, on erythropoietin treatment or taking iron supplementation, red cell concentrate (RCC) transfused within last 3 months. Patients currently on chemotherapy or have taken chemotherapy during last one year were also excluded. Patients with diluted marrow aspirate were excluded from the study.

Demographic and relevant clinical data was recorded. Bone marrow aspiration and trephine biopsies were taken from posterior superior iliac spine under local anesthesia and strict aseptic measures. 1-2ml of bone marrow aspirate was sucked out using 18 gauge bone marrow aspiration needle attached to 20ml syringe. Aspirate was examined for fragments and slides were prepared at bedside. These slides were stained with Leishman and Geimsa stain for cytological examination. Perls staining was done for intracellular and extracellular iron. The iron appears bright blue against red background. Bone marrow iron was graded into six levels according to Bain, *et al*, Bone marrow Pathology.[9] These six grades were numbered in increasing order of iron stain, starting from 0=absent, 1= trace, 2=reduced, 3=present, 4=increased, 5=markedly increased very large clumps with extra-cellular iron.

Blood samples (3ml) were taken in EDTA tubes and analyzed immediately for complete blood count using Sysmex Kx21. Peripheral smear were examined manually. Approximately 3ml blood was collected in gel tubes from each patient for analysis of soluble serum transferrin receptor (sTfR), ferritin and C-reactive protein (CRP). Centrifugation was done at 3500 rpm for 3 minutes to separate the serum. Sera were kept at 2-8^{o}C, in case there was any delay in analysis. Quantitative analysis of sTfR and CRP was done using Roche Cobas C-501 random access chemistry auto-analyzer. Ferritin was analyzed using electrochemiluminescence immunoassay on Roche Cobas e-411 auto-analyzer.

Anemia was defined as Hb <11.5 g/dL in females and <13.5 g/dL in males. 43 (78%) were anemic and 12(21%) were non-anemic10. Based on hemoglobin (Hb) level, patients were labeled with anemia or no-anemia. Patients with anemia were further divided into 3 groups, iron deficiency anemia (IDA), anemia of chronic disorder (ACD) and mixed anemia (IDA+ ACD). Those patients, who had absent or trace/reduced bone marrow iron; were labeled with iron deficiency anemia (IDA). Patients who had raised CRP and/ or TLC were categorized under anemia of chronic disorder. Patients who had both were classified under mixed anemia (IDA+ACD) group. Transferrin index was calculated by dividing sTfR in mg/dL by log of ferritin in ng/mL. Sensitivity, specificity, positive predictive value, negative predictive value, likelihood ratio and diagnostic accuracy for serum ferritin, sTfR and transferrin index were calculated using respective formulas. Statistical Package for the social sciences (SPSS) version 23 was used to analyze data. Receiver Operator Characteristic (ROC) graph was made for the area under the curve (AUC) of the studied parameters to reflect the bone marrow iron stores. One-way ANOVA test was used to observe the difference of mean of three studied parameters between the groups i.e. IDA, ACD, Mixed anemia group. The *p*-value of ≤0.05 was considered.

RESULTS

Out of 55 enrolled patients, 38 (69%) were males and 17 (31%) were females. The mean age was 45.82 ± 21.56 years. Minimum and maximum hemoglobin level was recorded to be 5.6 g/dL and 16.5 g/dL with the mean of 10.70 ± 2.73 mg/dL. There were 16 cases of pure IDA, 18 cases of pure ACD and only 9 cases of mixed anemia.

Out of 16 patients with IDA, 9 (56.2%) had totally absent, 5 (31.2%) had trace and only 2 (12.5%) reduced iron in the bone marrow. Among 9 cases of mixed anemia, 2 (22.2%) had trace, 5 (55.5%) had reduced and 2 (22.2%) had increased stainable iron in the bone marrow (Table-I). None of ACD case had reduced

marrow iron. Serum ferritin was 28%sensitive but 100% specific for reflecting reduced bone marrow iron stores. However, sTfR had sensitivity: 60% and specificity: 96.6% (Table-II). The sensitivity and specificity of the transferrin index was calculated to be 64% and 83% respectively. Among three studied parameters, sTfR had diagnostic accuracy of 80%, transferring index (74.5%) and ferritin (67.2%) (Table-II).

Table-I: Ferritin, Soluble Serum Transferring Receptor (sTfR), Transferring Index [sTfR/log ferritin] values in the study participants

Parameters (n=55)	Bone Marrow iron reduced/ trace/absent	Bone marrow iron stores present/ increased
Ferritin reduced	7 (12.7%)	-
Ferritin WNL*/ increased	18 (32.7%)	30 (54.5%)
Soluble serum transferrin receptor increased	15 (27.2%)	1 (1.81%)
Soluble serum transferrin receptor WNL*/reduced	10 (18.1%)	29 (52.7%)
Transferrin index >2	16 (29%)	5 (9%)
Transferrin index <2	9 (16.3%)	25 (45.4%)

**WNL= within normal limits*

The receiver operator characteristic (ROC) graph was made to see the area under the curve (AUC) of the studied parameters to reflect the bone marrow iron stores. Transferrin index (AUC=0.908) showed maximum accuracy, followed by ferritin (AUC=0.884) and sTfR (AUC=0.879) (Figure-1 & 2).

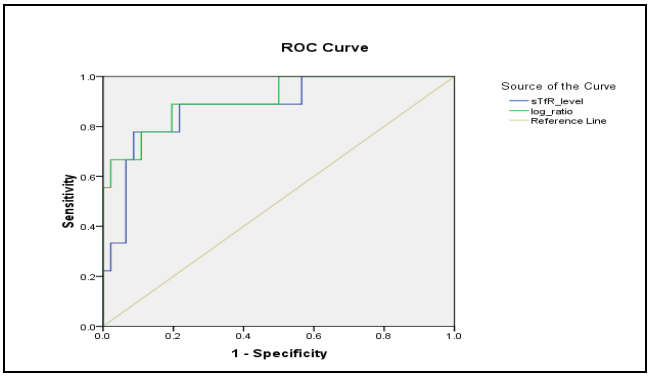

Figure-1: ROC of sTfR&transferrin index (AUC of sTfR = 0.879, AUC of Tansferrin index = 0.908).

Statistically significant difference in the mean of sTfR (p=0.001) and tranferrin index (p=0.006) was observed between groups i.e. IDA, ACD and mixed deficiency anemia. However, for serum ferritin this difference was not statistically significant (p=0.323) Table-III & IV.

DISCUSSION

In the clinical practice, the most commonly encountered iron deficiency state is associated with the underlying inflammatory conditions. This usually manifests as presence of mixed patterns of iron variables. It is a daily dilemma especially in tertiary care hospitals, to differentiate between the anemia of chronic disorder and iron deficiency anemia with underlying inflammatory cause.

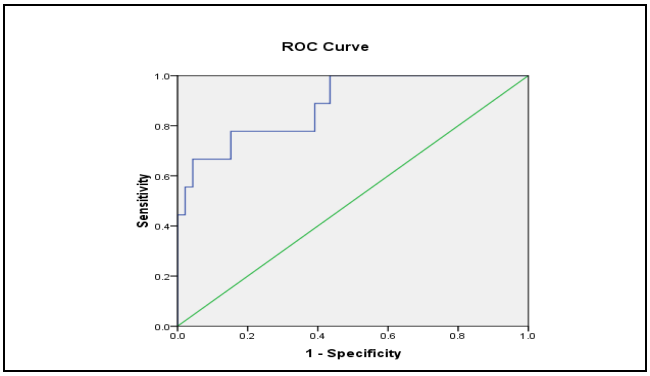

Figure-2: ROC graph of ferritin (AUC= 0.884).

In our study serum ferritin was found to be less sensitive (28%) but more specific (100%) for reflecting reduced bone marrow iron stores as compared to sTfR (sensitivity: 60%, specificity: 96.6%). sTfR had highest likelihood ratio of positive test i.e.[15] It means that the probability of having iron deficiency is very high when sTfR is raised. Among three studied parameters, sTfR had highest diagnostic accuracy of 80%, followed by transferring index (74.5%) and ferritin (67.2%).

Alan E. Mast and coworkers studied the clinical utility of sTfR in different populations and found out that diagnostic sensitivity and specificity of sTfR (at a diagnostic cutoff of >2.8 mg/L) were 92% and 84%, respectively, with a positive predictive value of 42% in anemic population.[11] They concluded that in patients with no anemia, sTfR does not provide any additional information over ferritin but it may be useful in patients with anemia and co-morbids when used conjunction to routine investigations. Their findings were in line with our findings. Skikne *et al* did a research on 145 patients with anemia and concluded that by using all three parameters in combination, the ability to detect the patients of IDA increased, from 41% (ferritin alone) to 92% (ferritin, sTfR and transferrin index). Thus, use of sTfR and the transferrin index improves detection of IDA, particularly in situations where routine markers provide equivocal results.[12]

In a study conducted by Junca *et al*, it was found that all the patients with iron deficiency anemia had a raised Serum TfR values. In contrast to our findings, they concluded that in patients having anaemia of chronic disease, sTfR cannot be accurately used to assess

Table-II: Sensitivity, specificity, positive predictive value and negative predictive value of serum ferritin, stfr& transferrin index.

Diagnostic Parameters	Ferritin	Soluble serum Transferrin Receptor (sTfR)	Transferring index (sTfR/log ferritin)
Sensitivity=True Positive/(True Positive +False Negative)	28%	60%	64%
Specificity=True Negative /(True Negative +False Positive)	100%	96.6%	83%
Positive Predictive Value= True Positive/(True Positive+ False Positive)	100%	93.5%	76%
Negative Predictive Value= True Negative/(True Negative +False Negative)	62.5%	74.4%	73%
Likelihood ratio for positive test= Sensitivity/1-specificity	-	15	3.76
Likelihood ratio for negative test= Specificity/1-sensitivity	1.38	2.4	2.3
Diagnostic accuracy	67.2%	80%	74.5%

Table-III: Comparison of Serum Ferritin, sTfR and transferrin index among various groups.

Parameters	Non anemic (n=12)	Iron deficiency anemia (IDA)(n=16)	Anaemia of chronic disease (ACD)(n=18)	IDA+ACD(n=9)	*p*-value
Serum Ferritin levels	377.5 ± 651.1	186.6 ± 366.8	502.3 ± 533.0	458.2 ± 455.9	0.323
sTfR	3.1 ± 1.1	7.9 ± 6.2	3.0 ± 1.2	5.1 ± 2.8	0.001
Transferrin index	1.6 ±0.9	6.7 ± 8.4	1.2 ± 0.6	2.4 ± 1.7	0.006

Table-IV: Post Hoc Tukey's Test for intergroup comparison.

Group Comparison	Non Anaemic Vs. IDA	Non AnaemicVs. ACD	Non Anaemic Vs. IDA+ACD	IDAVs. IDA+ACD	IDAVs. ACD	ACDVs. IDA+ACD
Transferrin index	0.020	0.900	0.900	0.100	0.006	0.900
sTfR	0.006	1.00	0.500	0.200	0.001	0.400

the iron levels due to its lower sensitivity and specificity as compared to the bone marrow aspiration and Perls staining, which remains the gold standard.[13] Similarly, in India a case control study was conducted on patients with anemia along stage 5 CKD, they conclude that owing to complexity of iron metabolism in CKD, sTfR cannot be used as a reliable marker of iron deficiency anemia.[14]

Harms *et al*, concluded that in cases of complicated anaemias, there is a limited validity of the commonly used biomarkers to assess iron metabolism. For the accurate differentiation of anaemia due to inflammation, sTfR derived indices should be employed rather than measuring sTfR alone.[15] Van *et al*, however showed contradictory results and revealed that ferritin and sTfR/ferritin ratio has significant predictive values for the differentiation of iron deficiency anaemia from other causes of anaemia. He further suggested that ferritin was the only independent and significant predictor of iron deficiency anemia with a cut-off point of 32 µg/l (sensitivity 79.2%, specificity 96.9%).[16]

In our study ROC analysis for serum ferritin showed that AUC was 0.884, for sTfR is 0.879, and of tansferrin index was 0.908. This showed that sTfR reflects bone marrow iron stores better than the serum ferritin levels but transferrin index remained the accurate marker. By definition from WHO, a low serum ferritin is described when it is <15 µg/l in adults and <12 µg/l in children. A level of 35 µg/l has been set as the most accurate with 92% sensitivity and 98% specificity.[17] Serum ferritin levels are affected by various factors including the physical and demographic variables. Particular importance is given to old age, obesity and concomitant comorbid conditions which are all interlinked and the most common features of patients having inflammatory conditions leading to iron deficiency anemia.[18]

Remacha *et al*, studied the differentiation of iron deficiency anaemia having features of inflammatory disease using sTfR levels and concluded that sTfR yield a sensitivity of 92% and specificity of 81% when the cut-off point used was 4.7 mg/L.[19] Apriyanti *et al*, studied the diagnostic accuracy of serum ferritin levels in detecting iron deficiency anaemia in children under five years of age and concluded that if <32.4 µg/l was used as a cut off value then serum ferritin had a sensitivity of 62.1% and specificity of 50.8%.[20] Another meta-analysis revealed that both sTfR and sTfR index showed a significant odds ratio (22.9, 95% confidence interval, 9.6-55.0 and 9.5, 95% CI, 5.0-18.1 respectively). The sensitivity was 86%, specificity was 75% with an AUC value of 0.91221.

Seema *et al*, in their study concluded that the diagnostic accuracy of sTfR was 85.4% which was comparatively lower than sTfR- serum ferritin index (91.57%) and higher than serum ferritin alone (75.09%)

in cases of iron deficiency anaemia. Where as in cases of anaemia of chronic disease the diagnostic accuracy of sTfR was higher (91.03%) than both the sTfR-Serum ferritin index (89.86%) and serum ferritin (79.71%).[22] Ejaz *et al*, in his study showed that the positive and negative predictive value of sTfR was 100% in cases of iron deficiency anaemia and in anaemia of chronic disease it was 100% and 74.1% respectively. He also concluded that if cut off of 90 ng/ml was used for serum ferritin levels then it can effectively exclude cases of iron deficiency anaemia especially those having other chronic inflammatory conditions.[23]

LIMITATION OF STUDY

It's a small, single center study in which few biochemical parameters were studied. For better analysis, large scale prospective study should be done.

CONCLUSION

sTfR and Transferrin index has advantage over serum ferritin alone in predicting the bone marrow iron stores. sTfR has good sensitivity (60%), specificity (96.6%), positive predictive value (93.5%) and highest positive likelihood ratio (15) and diagnostic accuracy (80%). sTfR and transferring index are superior to the old marker i.e. serum ferritin for differentiating iron deficiency anemia from anemia of chronic disease.

Conflict of Interest: None.

Authors' Contribution

TA: Designed the study, collection & analyzed the data, wrote initial draft of paper, AH: Performed the bone marrow aspiration prepared & analyzed the study, AS: Designed the study, N: Studied and diagnosed the bone marrow cases, revised and edited the preper, NS: Independently reviewed the bone marrow cases, FM: Data collection and interpretation reviewed & edited the paper.

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