Study of Soluble Transferrin Receptor and Soluble Transferrin Receptor - Log Ferritin Index in Differentiating between Iron Deficiency Anemia, Anemia of Chronic Disease and Combined Anemias

S Riaz Mehdi[1], Farhana[2], Noorin Zaidi[3], Imran Rizvi[4]

ABSTRACT

Introduction: Anemia can be defined as reduced oxygen carrying capacity of blood due to various reasons and iron deficiency is the commonest cause of anemia. However it is not easy to distinguish between iron deficiency anemia, anemia of chronic disease and combined iron deficiency anemia and anemia of chronic disease (COMBI anemia) using the present iron profile parameters alone. Bone marrow being an invasive procedure there is a need to find better non invasive methods. Hence this study was undertaken.

Material and methods: This was a prospective observational study and the study population was divided into the three respective anemia groups. All the included patients were evaluated clinically and routine investigations were done along with iron profile. Subsequently serum soluble transferrin receptor (sTfr) evaluation was done by ElISA method and index of sTfr and log ferritin was calculated. Statistical ananlysis was performed.

Results: Values of sTfr and sTfr- log ferritin index showed a significant difference among the three groups. The sTFR of iron deficiency anemia patients was significantly higher as compared to anemia of chronic disease ($p<0.001$) and combined anemia ($p<0.001$). Patients with combined anemia also had a significantly higher sTFR as compared to the anemia of chronic disease ($p<0.001$). The sTfr- log ferritin index of iron deficiency anemia patients was significantly higher as compared to anemia of chronic disease ($p<0.001$) and combined anemia ($p<0.001$). Patients with combined anemia also had a significantly higher index as compared to the anemia of chronic disease ($p<0.001$).

Conclusion: Thus it was concluded that these two parameters, i.e. sTfr and sTfr- log ferritin index, have a promising role in differentiating between the three types of anemias.

Keywords: Iron Deficiency Anemia, Anemia of Chronic Disease, COMBI - Anemia, sTfr, sTfr-log Ferritin Index

INTRODUCTION

Anaemia is defined by the World Health Organisation as haemoglobin (Hb) < 12 g/dl in women and Hb < 13 g/dl in men[1]. Iron deficiency is the most common cause of anemia worldwide. Iron deficiency anemia (IDA) afflicts a subset of the two billion people worldwide who are nutritionally iron deficient.[2] Classic iron deficiency (ID) does not represent a challenge for the laboratory investigations and physicians. The anemia that accompanies infection, inflammation, and cancer, commonly termed anemia of chronic disease (ACD), features apparently normal or increased iron stores. However, 20% of these patients have iron-restricted erythropoiesis (functional ID), an imbalance between the iron requirements of the erythroid marrow and the actual iron supply.[3]

Differentiation between IDA and ACD can be made by assessment of stainable iron in bone marrow. However, bone marrow examination is an invasive procedure resulting into inconvenience and discomfort to the patient. Red cell indices and iron parameters such as total iron binding capacity (TIBC) show considerable overlap. In general, IDA is associated with a serum ferritin value below 20 ng/ml whereas a serum level above 100 µg/L excludes iron deficiency in majority of cases. Serum ferritin being an acute phase reactant increases nonspecifically in inflammatory conditions despite the presence of iron deficient stores and values between 30 and 100 µg/L fall in the diagnostic gray zone.[4]

Serum soluble Transferrin Receptor (sTfR) is a truncated form of the Transferrin receptor, without its transmembrane and cytoplasmic domains, and circulates bound to transferrin.[5] It is found to be present on erythroblasts in bone marrow and many other cells. The number of sTfR reflects the cellular requirements for iron, and varies with the function and the morphological development of each cell type. Measurement of sTfR is a new marker of iron metabolism that reflects body iron stores and total erythropoiesis.[6] In IDA the numbers of sTfR increase significantly, whereas the serum concentration of sTfR is an indicator of iron supply available for erythropoiesis; sTfR reflects erythropoiesis and inversely correlates with the amount of iron available for erythropoiesis.[7] It has also been proven to be unaffected by acute phase response like ferritin.[8] Thus sTfR, which is not influenced by chronic or acute inflammation; could be a more reliable index in diagnosing IDA in patients with chronic disease.[9] Though Serum sTfR concentrations are elevated in the IDA and COMBI anemias (ACD with IDA) group.

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To distinguish IDA from COMBI on the basis of iron status is still difficult. However, the detection of iron deficiency in COMBI patients is very useful for the initiation of replacement therapy.[10] Apart from sTfR, some other derived variables like ratio of serum Transferrin receptor to the log Ferritn which is known as sTfR-F Index has also shown to have some promising value in diagnosis of anemia of chronic disease having inflammatory bowel disease (IBD).[11]

Therefore the present study was undertaken to differentiate between iron deficiency anemia, anemia of chronic disease and COMBI anemia using sTfr and sTfr-F Index.

MATERIAL AND METHODS

Study design and setting: This was a hospital based prospective observational study. The study period was between august 2014 to august 2017. The study was carried out at a tertiary care centre located in northern India. A written and informed consent was obtained from each and every patient before enrolment. The study was approved by the institutional ethical committee.

Inclusion criteria with definitions

All cases of anemia attending Out Patient Department/Ward in medicine, obstetrics and gynaecology and paediatrics departments were included in the study and then divided into three groups based on their history and investigative findings. These groups were pure iron deficiency anemia, anemia of chronic disease and combined iron deficiency and anemia of chronic disease (COMBI anemia) respectively. Anemia was taken as haemoglobin concentration less than 12 in females and children and less than 13 in males. Microcytosis was defined as mean corpuscular volume less than 75.

Iron deficiency anemia was defined as anemia with microcytic hypochromic picture and serum ferritin levels less than 20 ng/ml and total iron binding capacity more than 450 microgram/dl.

Anemia of chronic disease was defined as anemia with serum ferritin and total iron binding capacity in normal range with or without microcytosis, and a history suggestive of chronic illness

Combined anemia was defined microcytic blood picture with ambiguous results of serum iron profile and suggestive history.

Exclusion criteria

Cases of macrocytic blood picture, haemolytic anemia, aplastic anemia were not included in the study. Patient's denying consent were also excluded from the study.

Patient's evaluation: All the patients underwent detailed clinical history and physical examination. Patients were asked about onset, course and duration of illness. History of fatigue, malaise, increasing pallor, shortness of breath, bleeding from any site, worm infestations, menstrual and family history were recorded on a predesigned Performa. Physical examination to look for pallor, icterus, edema, lymph nodes, heptomegaly and splenomegaly was done in each subject. History of any chronic illness or symptoms suggestive of chronic illness were specially sought for.

Investigations

Complete blood count was obtained for all samples using SYSMEX 800i (5 part). Hemoglobin, total red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, hematocrit, total leukocyte count, differential leukocyte count and platelet count were noted from there. Serum Iron and TIBC were estimated by Ferrozine method (crest biosystems). Serum ferritin was determined by Calbiotech, Inc. (CBI) Ferritin SA ELISA Kit. **Soluble transferrin receptor** (sTFR) estimation was done by Human sTFR ELISA kit manufactured by Bio Vender. Transferrin log ferritin index was measured by dividing the value of sTFR by log value of serum ferritin.

Treatment

Treatment was given in the respective departments depending on the diagnosis made.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS version 16.0 (Chicago, IL, USA). Categorical variables were expressed as percentages. Continuous variables were expressed as median, inter-quartile range (IQR) as well as mean ± standard deviation (SD). The categorical variables were compared using the Chi-square test. The continuous variables were subjected to the Shapiro-Wilk test to test the normality. Since most of the continuous variables showed a departure from normality non-parametric tests were used to compare them. The Kruskal-Wallis test was used to compare continuous variables in more than 2 groups. The post-hoc analysis was done further between to compare each type of anemia, for post hoc analysis Mann-Whitney U test was used. P value of <0.05 was taken as significant, but for post-hoc analysis Bonferroni correction was applied and a p value <0.017 was taken as significant.

RESULTS

Baseline characteristics

The baseline characteristics of 75 patients with anemia are shown in Table-1. The mean age of patients was 25.19 ± 15.86 years with a median of 25 years. Fifty four (72%) patients were females. The mean hemoglobin was 6.68 ± 1.00 g/dl with a median of 6.8 g/dl. The median MCV, MCH, MCHC were 70, 26, and 29 respectively. The mean total leukocyte count was 7130.67 ± 1123.22/ mm^3 (median = 7500). The median serum iron, serum ferritin and total iron binding capacity were 29.00, 70.40, and 375.00 respectively. Twenty five patients were of iron deficiency anemia, 25 patients were of anemia due to chronic disease and 25 patients were having anemia due to iron deficiency as well as anemia due to chronic disease. The mean transferrin index was 10.56 ± 9.60 with a median of 7.40. The median soluble transferrin receptor concentration was 3.93 with a mean of 6.40 ± 6.61.

Comparison of hematological parameters according to the type of anemia

The comparison of demographic and hematological parameters according to the type of anemia is shown in

S. No	Variable	Value
1.	**Age** Mean ± SD Median (IQR)	 25.19 ± 15.86 25 (22)
2.	**Sex** Males (%) Females (%)	 21 (28%) 54 (72%)
3.	**Haemoglobin** Mean ± SD Median (IQR)	 6.68 ± 1.00 6.8 (1.4)
4.	**MCV** Mean ± SD Median (IQR)	 72.22 ± 9.46 70 (13.8)
5.	**MCH** Mean ± SD Median (IQR)	 26.31 ± 2.28 26 (3)
6.	**MCHC** Mean ± SD Median (IQR)	 29.94 ± 3.45 29 (6)
7.	**Total leukocyte count** Mean ± SD Median (IQR)	 7130.67 ± 1123.22 7500 (2000)
8.	**Platelet count** Mean ± SD Median (IQR)	 2.978 ± 0.48 3.00 (1.00)
9.	**Serum Iron** Mean ± SD Median (IQR)	 30.65 ± 7.27 29 (3)
10.	**Serum Ferritin** Mean ± SD Median (IQR)	 135.88 ± 130.14 70.40 (261.50)
11.	**TIBC** Mean ± SD Median (IQR)	 315.95 ± 95.51 375.00 (190)
12.	**Diagnosis** Iron deficiency Anaemia of chronic disease Mixed	 25 (33.3%) 25 (33.3%) 25 (33.3%)
13.	**Transferrin index** Mean ± SD Median (IQR)	 10.56 ± 9.60 7.400 (18)
14.	**sTFR** Mean ± SD Median (IQR)	 6.40 ± 6.61 3.93 (11.12)

SD= standard deviation; IQR= Inter quartile range; MCV= mean corpuscular volume; MCH= mean corpuscular haemoglobin; MCHC= mean corpuscular haemoglobin concentration; TIBC= total iron binding capacity; sTFR= soluble Transferrin receptor

Table-1: Baseline characteristics of 75 patients with anemia

Table-2. No significant difference was noted in age and sex distribution amongst different type of anemia. There was no significant difference in hemoglobin level, total leukocyte count and platelet count amongst the three groups.

A significant difference was noted in different red cell indices namely MCV, MCH and MCHC amongst the three groups (P<0.001). Post hoc analysis with Mann-Whitney U test was conducted with a Bonferroni correction applied, resulting in a significance level set at p < 0.017. The median (IQR) MCV, MCH and MCH of iron deficiency anemia was significantly lower than anemia of chronic disease (P <0.001). On comparing iron deficiency with combined anemia MCV was found to be significantly lower (P<0.001), however no difference was noted in MCH, and MCHC. (Table-2)

A significant difference was also noted in the iron profile of the 3 groups, serum iron (p=0.012), serum ferritin (p<0.001) and TIBC (p<0.001). On post-hoc analysis serum ferritin was significantly found to be lower in patients of iron deficiency anemia as compared to anemia of chronic disease (p<0.001); TIBC of iron deficiency anemia patients was found to be significantly higher than anemia of chronic disease (p<0.001); however no significant difference was noted in the iron level in iron deficiency anemia and anemia of chronic disease. On comparing iron profile in iron deficiency anemia with combined anemia a significant difference was noted in all the three parameters namely serum iron (p=0.008), serum ferritin (p<0.001) and TIBC (p=0.003). (Table-2)

Comparison of soluble transferrin receptor according to the type of anemia

The sTFR levels showed a significant difference in the three groups (p<0.001). The median (IQR) sTFR of iron deficiency anemia, anemia of chronic disease and combined anemia was 13.63 (4.81), 0.79 (0.16), and 3.93 (2.21) respectively. The sTFR of iron deficiency anemia patients was significantly higher as compared to anemia of chronic disease (p<0.001) and combined anemia (p<0.001). Patients with combined anemia also had a significantly higher sTFR as compared to the anemia of chronic disease (p<0.001)

There was a significant difference in TI of all the 3 groups (p<0.001). Median (IQR) of iron deficiency anemia, anemia of chronic disease and combined anemia was 21 (7.8), 1.1 (0.3), and 7.4 (1.0) respectively. The TI of iron deficiency anemia patients was significantly higher as compared to anemia of chronic disease (p<0.001) and combined anemia (p<0.001). Patients with combined anemia also had a significantly higher TI as compared to the anemia of chronic disease (p<0.001)

DISCUSSION

In present study, we evaluated two markers – soluble transferrin receptor (sTfR) and Transferrin index among three different patient groups. sTfR, a cell surface glycoprotein mediates cellular uptake of iron by binding the iron carrier-protein transferrin (Tf) and unlike ferritin is unaffected by inflammatory conditions as evidenced in ACD.[12] Ratio of sTfR to log serum ferritin is termed as Transferrin index.

No significant difference was noted in age and sex distribution, hemoglobin level, total leukocyte count and platelet count amongst the three groups. Although total leucocyte count (TLC) is also considered to be an inflammatory marker [13,14] indicative of chronic conditions, however, this was not the case in our study.

A significant difference was also noted in the iron profile of the 3 groups, serum iron (p=0.012), serum ferritin (p<0.001) and TIBC (p<0.001), however no significant difference was noted in the iron level in iron deficiency anemia and anemia of chronic disease. Similar to results of present study, Jain

Variable	IDA N=25	ACD N=25	Mixed N=25	P value overall *	IDA Vs. ACD**	IDA Vs. Mixed**	Mixed Vs. ACD**
Age							
Mean ± SD	26.84 ± 12.53	24.240 ± 19.83	24.50 ± 14.87	0.482	0.248	0.515	0.515
Median (IQR)	30.00 (21.00)	15.00 (29)	27.00 (20.5)				
Sex							
Males (%)	04 (16%)	10 (40%)	7 (28%)	0.168	0.06	0.31	0.37
Females (%)	21 (84%)	15 (60%)	18 (72%)				
Haemoglobin							
Mean ± SD	6.94 ± 1.25	6.46 ± 0.546	6.63 ± 1.06	0.156	0.045	0.259	0.562
Median (IQR)	7.00 (2.3)	6.80 (1.00)	6.80 (1.7)				
TLC							
Mean ± SD	7076 ± 1151.91	7252 ± 983.24	7064 ± 1254.29	0.770	0.550	0.976	0.515
Median (IQR)	7500 (1950)	7500 (1300)	7500 (2200)				
MCV							
Mean ± SD	63.08 ± 6.19	83.54 ± 2.91	70.05 ± 2.00	<0.001	<0.001	<0.001	<0.001
Median (IQR)	64.00 (12.3)	82.00 (5.00)	70.00 (1.9)				
MCH							
Mean ± SD	24.62 ± 1.76	28.71 ± 1.07	25.60 ± 1.50	<0.001	<0.001	0.093	<0.001
Median (IQR)	25.00 (2.3)	28.50 (1.4)	25.00 (6.00)				
MCHC							
Mean ± SD	27.20 ± 1.91	33.96 ± 1.02	28.64 ± 2.36	<0.001	<0.001	0.038	<0.001
Median (IQR)	27.00 (3.00)	34.00 (2.00)	28.00 (3.00)				
Platelet count							
Mean ± SD	2.91 ± 0.45	3.04 ± 0.49	2.98 ± 0.51	0.552	0.272	0.617	0.576
Median (IQR)	3.00 (0.8)	3.00 (0.7)	3.00 (1.00)				
S. Iron							
Mean ± SD	28.24 ± 1.98	28.51 ± 1.74	35.21 ± 11.12	0.012	0.643	0.008	0.015
Median (IQR)	28.00 (3.00)	28.00 (2.50)	30.00 (11.8)				
S. Ferritin							
Mean ± SD	5.01 ± 1.68	295.96 ± 49.82	106.66 ± 65.66	<0.001	<0.001	<0.001	<0.001
Median (IQR)	5.80 (3.00)	302.00 (62.00)	70.40 (89.60)				
TIBC							
Mean ± SD	388.68 ± 15.90	183.68 ± 15.45	375.48 ± 14.55	<0.001	<0.001	0.003	<0.001
Median (IQR)	390.00 (19)	180.00 (31.00)	378.00 (30.00)				
Transferrin index							
Mean ± SD	22.47 ± 5.48	1.08 ± 0.21	8.14 ± 2.52	<0.001	<0.001	<0.001	<0.001
Median (IQR)	21.00 (7.8)	1.10 (0.30)	7.40 (1.00)				
sTFR							
Mean ± SD	14.50 ± 5.15	1.04 ± 0.72	3.66 ± 1.20	<0.001	<0.001	<0.001	<0.001
Median (IQR)	13.63 (4.81)	0.79 (0.16)	3.93 (2.21)				

SD= standard deviation; IQR= Inter quartile range; MCV= mean corpuscular volume; MCH= mean corpuscular haemoglobin; MCHC= mean corpuscular haemoglobin concentration; TIBC= total iron binding capacity; sTFR= soluble Transferrin receptor; IDA= iron deficiency; ADC= Anaemia of chronic diseases. * derived by Kruskal Wallis test, ** derived by Mann Whitney U test

Table-2: Comparison of various demographic and haematological parameters amongst the different types of anaemia

et al. (2010)[15] also did not find a significant difference in S. iron levels of ACD and IDA patients thereby questioning its validity as a discriminant. In present study we observed that S. ferritin levels of both ACD and COMBI groups to be significantly higher as compared to those in IDA, and this is the reason where S. ferritin levels fail to discriminate for iron deficiency. Similarly Punnonen et al. (1997)[16] and Yokus et al. (2011)[17] also highlighted the feature that S. ferritin levels fail to identify IDA in those with coexisting IDA and ACD.

The sTFR levels showed a significant difference in the three groups. The sTFR of iron deficiency anemia patients was significantly higher as compared to anemia of chronic disease and combined anemia. Patients with combined anemia also had a significantly higher sTFR as compared to the anemia of chronic disease. These findings are in agreement with the observations of Punnonen et al. (1997)[16] and Yokus et al. (2011)[17].

The TI of iron deficiency anemia patients was significantly higher as compared to anemia of chronic disease and combined anemia. Patients with combined anemia also had a significantly higher TI as compared to the anemia of chronic disease. Similar to results of present study Hanif et al.[18] reported 100% sensitivity as well as specificity for diagnosis of IDA whereas Aslam et al.[19] also reported 100% sensitivity and 80% specificity of TI for diagnosis of IDA.

CONCLUSION

Hence in light of the present study we can conclude that sTfr levels in conjunction with transferring index values can be used for differentiating between iron deficiency anemia,

anemia of chronic disease and COMBI-anemia.

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