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ORIGINAL ARTICLE

Efficacy of Soluble Transferrin Receptor in Diagnosis of Iron Deficiency Anemia in Chronic Kidney Disease in Children

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ABSTRACT

Background: Anemia is common complication in children with chronic kidney disease (CKD) mainly anemia of chronic disease (ACD), but iron deficiency anemia (IDA) is also common. Soluble transferrin receptor (sTfR) is important marker for IDA. This study aims to evaluate the utility of soluble transferrin Receptor-Ferritin Indices as markers of IDA and for differentiation ACD from IDA in childern with chronic kidney disease.

Methods: cross-sectional study was conducted at Department of Pediatric Nephrology at Zagazig University Hospital including 45 anemic children (19 ACD, 13 IDA and 13 mixed). The duration of the study ranges from 6 to 12 months, Soluble serum transferrin receptor and Soluble serum transferrin receptor index were measured.

Results: There was significant difference regard sTfR and sTfR index IDA group were significantly higher than other two groups then mixed and finally ACD were significantly lower.

Conclusion: sTfR value was useful tool for assessment of iron status in patients with CKD, however, they are at best complementary to the existing indices of serum ferritin and TSAT.

Keywords: Anemia, Anemia of Chronic Disease, Ferritin, Iron Deficiency Anemia, Soluble Transferrin Receptor.

INTRODUCTION

Patients of chronic kidney disease (CKD) suffer from different types of anemia as anemia of chronic disease (ACD) and iron deficiency anemia (IDA). In CKD patients, anemia may be due to decrease erythropoietin production by kidneys due to damage to the interstitial cells of the diseased kidney; RBC survival reduced by 30–60% also elevation of toxin production causing hemolysis of RBCs [1].

Subclinical inflammation is the main cause for ACD in patients of CKD. Iron deficiency anemia percentage is 25– 38% of children with anemia of CKD. The main causes of IDA in CKD patients are reduced intake, losses of blood during dialysis, reduced intestinal absorption of dietary iron, chronic inflammation, bleeding tendency which can cause bleeding, and erythropoiesis-stimulating agents (ESAs) which elevate requirement of iron [2].

Conventional laboratory tests of iron status can be used to diagnose iron deficiency, as transferrin saturation and serum ferritin. But these tests do not always reflect the iron status of CKD patients, as transferrin and Serum ferritin are affected by acute phase responses in inflammation. Also, Transferrin saturation (TSAT) varies because of serum iron level diurnal variation [3]. Discrimination between IDA, ACD and mixed anemia (coexisting ACD

and IDA) is a recurrent diagnostic problem, which is clinically common because inadequate iron supplementations [2].

Bone marrow examination is the gold standard for a diagnosis of iron deficiency. However, it is invasive and needs technical expertise, so that it cannot be done in clinical practice routinely. Recently, soluble transferrin receptor (sTfR) has been introduced as early and sensitive marker of iron deficiency. sTfR protein is a single polypeptide that can be detected in human serum, which derived from human transferrin receptor [4].

The concentration of sTfR is an indicator of iron status. Soluble transferrin receptor (sTfR) concentrations are inversely related to iron status. In Iron deficiency causes overexpression of transferrin receptor and sTfR levels, while iron repletion results in decreased sTfR levels. [5].

The study aimed to assess the utility of soluble transferrin Receptor-Ferritin Indices as markers of IDA and to identify ACD from IDA in patients with chronic kidney disease.

Methods

The present study was a cross sectional study conducted in Pediatric Nephrology Unit at Zagazig University Hospital and Clinical Pathology Department from October 2019 to October 2020. In this study 45 anemic children were included, known to have chronic kidney failure by laboratory investigation (urea, creatine, urine analysis,) abdominal ultrasound or renal biopsy.

Inclusion Criteria: All patients from 3 months to 12 years, diagnosed with chronic renal failure for 3 months or more, on dialysis or not, evaluated clinically and by laboratory parameters for anemia.

Exclusion criteria: Who had blood transfusion in the 4 weeks proceding the enrolment. A history of acute infection and inflammation in the 4 weeks preceding the enrolment.

Written informed consent was obtained from all participants, the study was approved by the research ethical committee of Faculty of Medicine, Zagazig University. The study was done according to The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

All patient subjected to the following: full history taking including age, sex, drug intake, nutritional history, symptoms suggesting anemia, medical history of kidney disease, history of hemodialysis or not.

Complete clinical examination, general look, activity, temperature, vital signs, systemic examination including neurological, respiratory, cardiovascular, abdominal, signs of anemia. Routine laboratory Investigations: CBC, Kidney function for BUN & serum creatinine, C-Reactive protein (CRP), Iron indices (ferritin, total iron binding capacity (TIBC), transferrin saturation (TAST). We defined anemia as a decrease of hemoglobin and/or hematocrit below the lowest limits of normal values for children age and sex according to the World Health Organization (WHO) (3).

A total of 45 patient with history of chronic kidney disease and anemia, admitted in nephrology unit, were divided in to 3 groups according to baseline investigation and sTfR to: Group A: patients with IDA (n=13) having a serum ferritin level of < 200 ng/ml (in patients on hemodialysis) and <100 ng/ml in non-hemodialysis CKD subjects and serum transferrin < 20% in defined (IDA), with sTfR above 5.2 (mg /L) and sTfR Index above 2.56 [5]. Group B: Patients with pure ACD (n=19) defined as those having a chronic disease and ferritin >200 ng/ml with sTfR Index below 1.5 and sTfR below 3 (mg/L) [2]. Group C: Patients with mixed type of anemia (n =13) ferritin >200 ng/ml with sTfR index above 1.5 and sTfR Above 3 (mg/L) [16].

Specimen collection: Four ml of peripheral blood (PB) were aseptically collected using sterile vacutainers and divided as follow Two ml (PB) was added to ethylene tetra acetic acid (EDETA) in a sterile vacutainer tube to do automated complete blood count (CBC). Two ml (PB) were dispended into a plain tube (no additive) for measuring serum ferritin level, total iron binding capacity (TIBC) and serum iron.

Specific investigation:

Soluble serum transferrin receptor: The kits used to assay the transferrin receptor in the sample of serum human, blood plasma, and other related tissue liquid. Serum was separated and frozen at -20C until analyzed. sTFR was measured with an enzyme- linked immunosorbent assay [6].

Test principle: The kit uses double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Transferrin in samples. Add soluble Transferrin receptor to monoclonal antibody Enzyme well which is pre- coated with Human soluble Transferrin receptor monoclonal antibody, incubation; then, add soluble Transferrin receptor antibodies labeled with biotin and combined with streptavidin-HRP to form immune complex ; then carry out incubation and washing again to remove the uncombined enzyme then add chromogen solution A, B, the color of the liquid changes in to the blue, and the effect of acid, the color finally becomes yellow. the chroma of color and the concentration of human substance soluble transferrin receptor of sample were positively correlated.

Specimen collection and storage: 2ml blood under sterile condition was collected left to clot at room temperature 10-20 mins centrifugation 20-min at the speed of 2000-3000 r.p.m. remove supernatant, if precipitation appeared, Centrifugal again and stred at -20 untill used.

STATISTICAL ANALYSIS

Data collected throughout history, basic laboratory investigations clinical examination, and outcome measures coded, entered, and analyzed using Microsoft Excel software. Data were then imported into Statistical Package for the Social Sciences (SPSS version 20.0) (Statistical Package for the Social Sciences) software for analysis. According to the type of data qualitative represent as number and percentage, quantitative continues group represent by mean ± SD, the following tests were used to test differences for significance; difference and association of qualitative variable by Chi square test (X2). Differences between quantitative multiple by ANOVA or Kruskal Wallis. a receiver operating characteristic (ROC) was used. P value was set at <0.05 for significant results & <0.001 for high significant result.

RESULTS

There was no significant difference among groups regard age or weight (Table 1). There was no significant difference among studied groups regard Urea, Cr, albumin, CBC, CRP, Retics and ESR (Table 2). There was significant difference among groups regard ferritin, TIBC , TSAT , s TfR , s TfR index (Table 3).

Figure (1) shows Flow chart showing criteria for diagnosing ACD, IDA and mixed groups. Figure (2) ROC curve show Sig AUC and Cutoff with sensitivity 88.8% and 92.0% respectively and specificity 95.0% and 97.5% respectively. Figure (3) ROC curve show Sig AUC and Cutoff with sensitivity 63.38% and 62.3% respectively and specificity 61.5% and 60.0% respectively. Figure (4) ROC curve show Sig AUC and Cutoff with sensitivity 97.5% and 95.0% respectively and specificity 95.0% and 98.3% respectively.

Table 1: Age, weight, and duration distribution among studied groups

	ACD (n=19)	Mixed (n = 13)	IDA (n =13)	Kruskal Walis	P
Age	8.24±2.68	8.3±2.9	6.38±2.12	1.592	0.215
(years) Median	5.4	5.5	4.3		
Weight	25.39±8.59	28.76±9.85	20.01±7.58	1.520	0.230
(kg) Median	16.4	19.2	15.3		
disease duration	5.11±3.35	4.90±3.11	7.38±3.52	2.328	.110
median	4.9	3.9	3.8		

ACD: anemia of chronic disease, IDA: iron deficiency anemia

Table 2: Urea, Cr, albumin, CBC, CRP, Retics and ESR distribution among studied group

	ACD (n =19)	Mixed (n =13)	IDA (n =13)	Kruskal Walis	P
Urea (mg/dl) median	53.1±21.24	48.61±21.56	71.49±42.41	1.149	0.327
	43	23.1	15.1		
Cr (mg/dl) median	3.46±1.74	4.65±3.12	3.96±2.74	0.494	0.614
	4.5	3.9	3.8		
Albumin (g/dl) Median	3.67±0.7	3.64±0.86	3.52±0.98	F=0.132	0.877
	2.1	2.4	1.7		
WBCs x10^3	9.48±3.19	8.60±2.57	9.27±2.97	0.231	0.795
HB (g\dl)	8.5±1.31	8.86±1.22	8.83±1.60	0.353	0.705
PLT x10^3	256.31±83.5	300.69±98.9	349.0±118.3	2.314	0.111
Ht (%)	25.24±4.27	26.38±3.35	27.23±4.08	0.997	0.378
MCV (fl)	71.12±16.2	75.46±7.56	73.23±8.29	0.493	0.614
MCHC(g\dl)	33.05±2.14	32.11±1.41	31.69±1.75	2.286	0.114
MCH (pg)	25.73±1.72	24.45±3.09	24.84±2.57	1.181	0.317
CRP (mg\l) median	18.26±17.69	9.75±8.98	16.35±16.08	0.563	0.574
	20	11.4	17.9		
Reticulocyte (%) Median	2.21±2.01	2.67±2.41	1.66±0.555	0.432	0.652
	4.3	2.8	1.4		
ESR (mm\h) Median	44.57±21.5	31.84±18.89	30.0±19.58	1.716	0.192
	38.7	29.1	26.7		

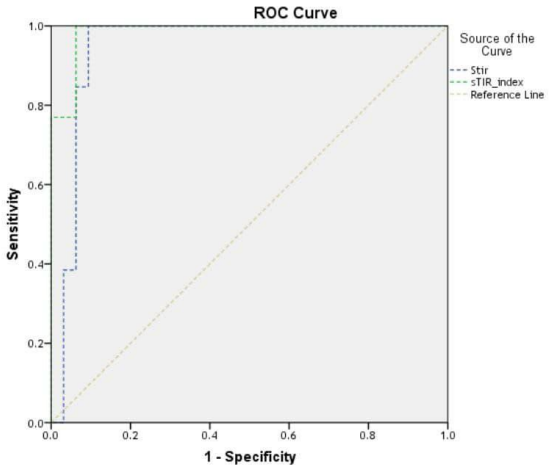
Cr: creatinine, WBC: white blood cell, plt: platelet , CRP: C- reactive protein, ESR: erythrocyte sedimentation rate.

Table 3: iron profile, s TFR and Index distribution among studied groups

	ACD (n =19)	Mixed (n =13)	IDA (n =13)	Kruskal Walis	P
FERRITIN (µg\l) (Median)	1395.73±1287.6	418.75±408.6	94.1±38.6	3.803	0.030*
	698.6	476.2	84.9		
TIBC (µg\dl) (Median)	234.26±80.62	226.15±75.0	232.76±78.1	0.044	0.957
	267.2	249.7	279.9		
TOTAL IRON	96.0±45.6*	64.47±26.8	45.82±24.85	6.612	0.003*

(µg\dl) (Median)	96	76.1	24.9		
TSAT	40.3±11.53	27.8±7.11	19.31±6.58	18.871	0.00**
(%) (Median)	38.9	15.5	12.7		
sTfR (µg\ml)	2.33±1.08	4.12±1.12	6.14±0.79	42.953	0.00**
(median)	1.43	1.8	3.7		
sTfR index	0.97±0.41	1.89±0.34	3.12±0.43	75.914	0.00**
(median)	0.8	1.1	2.4		

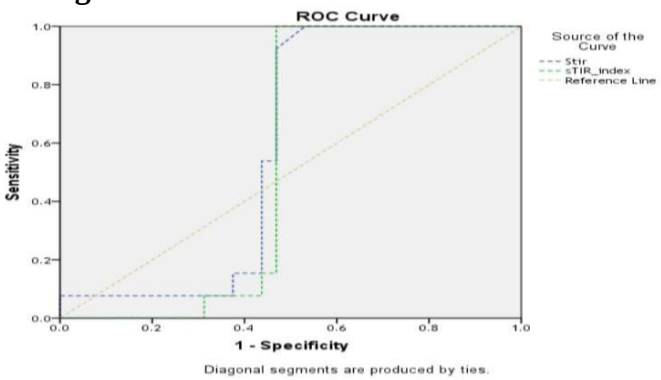
TIBC: total iron binding capacity, TSAT: transferrin saturation, STfR: serum transferrin receptor



ROC Curve for detection of IDA

Area Under the Curve					
Test Result Variable(s)	Area	Cutoff	P	95% Confidence Interval	
				Lower Bound	Upper Bound
sTfR	**.945**	**>5.2**	**0.00****	**.873**	**1.000**
sTfR_index	**.986**	**>2.56**	**0.00****	**.960**	**1.000**

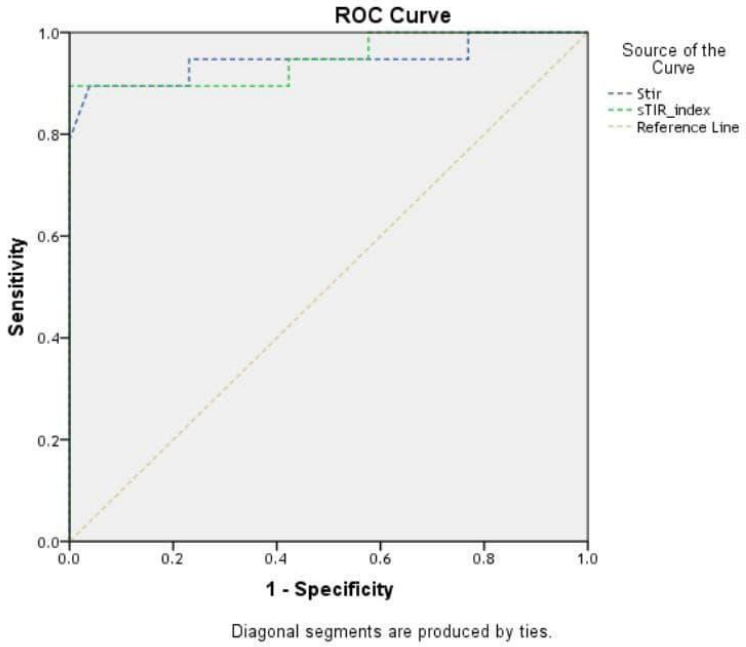
Fig 1: ROC curve for detection of IDA.



ROC Curve for detection mixed

Area Under the Curve					
Test Result Variable(s)	Area	Cutoff	P	95% Confidence Interval	
				Lower Bound	Upper Bound
s TfR	**.684**	**>3.95-<5.2**	**0.045***	**0.515**	**.853**
sTfR_index	**.646**	**>1.49-<2.56**	**0.048***	**0.576**	**.765**

Fig 2: ROC curve for detection of mixed anemia.



ROC Curve for detection ACD

Area Under the Curve					
Test Result Variable(s)	Area	Cutoff	P	95% Confidence Interval	
				Lower Bound	Upper Bound
s TfR	**0.945**	**<3.95**	**0.00****	**.864**	**1.000**
sTfR_index	**0.947**	**<1.49**	**0.00****	**.874**	**1.000**

Fig 3: ROC curve for detection of ACD.

DISCUSSION

The current study showed no significant difference among groups regard age, sex, duration of illness. The results of this study were in agreement with study of Gupta et al [7] as they reported that there was no significant difference among groups regard age, sex and duration of illness.

The current study showed that there was no significant difference among studied groups as regard urea, creatinine, and albumin. There was no significant difference among studied groups regard any items of CBC. There was no significant difference among studied groups as regard CRP, retics and ESR.

These results were in line with study of Margetic et al [8] as they found that there was no significant difference among studied groups regard any items of CBC and albumin.

In contrary with our results, study of Latif et al [2] as they reported that there was significant difference between their studied groups regarding items of CBC. The population of study were classified into two groups; Group 1, patients (30) who were having IDA and Group 2, patients (40) with ACD and selected control group.

In the study in our hands, ACD group were significantly higher than other groups regard Ferritin then mixed and finally IDA group were significantly lower, regard total Iron ACD group were significantly higher than other two groups with no significant difference between them, regard TSAT, ACD group were significantly higher than other groups then mixed and finally IDA group were significantly lower.

In this study, regarding sTfR and sTfR index,

IDA group were significantly higher than mixed group and finally ACD group were significantly lower.

Our results were supported by study of Latif et al [2] as they showed that Mean sTfR level was higher (4.81± 1.64 µg/ml) in IDA patients than (2.89±1.40 µg/ml) in ACD patients (p <0.0001). Mean ferritin level was 599.59± 449.15µg/L patients with ACD whereas 101.23±119.42 in patients with IDA (p<0.0001). It has been shown that, ferritin values elevated in IDA with CKD patients, may be due to chronic inflammation. Total iron binding capacity was higher in patients with ACD with sTfR ”3µg/ml in comparison to patients with IDA with sTfR<3µg/ml. Transferrin saturation level was significantly reduced in ACD patients with sTfR”3µg/ml as compared to patients with IDA with sTfR<3µg/ml. sTfR and ferritin indices between group 1 (IDA) and group 2 (ACD) shows mean sTfR:logSF level was significantly (P<0.001) high in group 1 (2.71±1.13) in comparison to group 2 (1.08±0.54). Mean log sTFR index was also significantly higher (P<0.05) in group 1 (0.001±0.0008) compared to group 2 (0.013±0.012).

Regarding Jain et al [9], mean ferritin level was significantly higher in patients with ACD than patients with IDA. In this study mean level of ferritin was (222.33±134.2) ìg/L in patients with ACD whereas (4.91±2.6) ìg/L in patients with IDA (P <0.001). While mean sTfR was significantly higher in patient with IDA (10.56±4.3) than in ACD patient (2.59±1.9) (P <0.001). Mean sTfR index in patient of ACD (1.05±0.54) and (3.35±0.38)

Peterson et al [10] reported that sTfR index was lower in ACD but elevated in IDA and patient with mixed (IDA and ACD). These studies supported sTfR index was a helpful tool to detect IDA in complicated type of anemia [11].

However, a recent meta-analysis by Infusino et al [12] showed that the sTfR index was no better than sTfR in identifying IDA in the presence of confounding condition.

In recent years, soluble transferrin receptor (sTfR) has been introduced as a sensitive, early, and valuable marker of iron depletion. sTfR is a truncated form of the transferrin receptor present on erythroblasts in bone marrow and many other cells. sTfR concentration is not affected by inflammation or infection rather in conditions where iron deficiency co-exists with ACD, sTfR raises secondary to underlying iron deficiency. Moreover, sTfR levels also reflect the rate of erythropoiesis, So, its specificity decreases as a sole marker of iron deficiency. Synthesis of transferrin receptor and the iron storage protein ferritin are reciprocally linked to cellular iron content. Thus, because of this reciprocal relationship between sTfR and serum ferritin, the sTfR /ferritin ratio reflects the iron status over the entire range [14].

The present study showed that as regard ROC Curve for detection of IDA, Sig AUC and Cutoff with sensitivity 88.8% and 92.0% respectively and specificity 95.0% and 97.5% respectively. As regard ROC Curve for detection mixed, Sig AUC and Cutoff with sensitivity 63.38% and 62.3% respectively and specificity 61.5% and 60.0% respectively. As regard ROC Curve for detection ACD, Sig AUC and Cutoff with sensitivity 97.5% and 95.0% respectively and specificity 95.0% and 98.3% respectively.

Gupta et al [7] showed that the cut-off level of sTfR at its maximum sensitivity of 63.6% and specificity of 64.8% was 3 with a PPV of 59% and NPV of 69%. The cut-off value of serum ferritin at its maximum sensitivity (67.3%) and specificity (67.6%) was 195 with a PPV of 27% and NPV of 38%. The cut-off level of sTfR index at its maximum sensitivity (71.8%) and specificity (62%) was 1.39 with a PPV of 62% and NPV of 70%. The cut-off value of transferrin saturation at its maximum sensitivity (61.8%) and specificity (62%) was 28.5 with a PPV of 32% and NPV of 44%. The cut-off level of total iron binding capacity with its maximum sensitivity (52.7%) and specificity (67.6%) was 272.5 with a PPV of 59% and NPV of 69%.

In the study of Margetic et al [8], ROC analysis revealed the differential power of the

sTfR and sTfR index in the assessment of status of iron calculated on the basis of ferritin and TSAT values. For sTfR concentration, an AUC of 0.890 was obtained with the best in combine with diagnostic sensitivity (81.8%) and specificity (90.5%) at a cutoff of 1.51 mg/L. The sTfR index revealed increase discriminating power in evaluating the iron status of anemia in CKD patients (AUC ¼ 0.970), with the best combination of diagnostic sensitivity (90.9%) and specificity (97.6%) at a cutoff of 0.969.

Furthermore, Suega et al [15], revealed that AUC (area under curve) for sTfR was 0.77 with p = 0.028 (95% CI 0.55-0.99). The cut-off value, at its maximum sensitivity of 83.3% and specificity of 67.2%, was 0.71. The TfR-F index has larger AUC, which is 0.85, with p = 0.004 (95% CI 0.69-1.00). The cut-off value, at its best sensitivity of 83.3% and specificity of 81.2%, was 0.33. The TfR-F index was superior compared to sTfR. When sTfR and TfR-F index were used in combination to determine the existence of IDA in regular hemodialysis patients, it was found that they carry the largest AUC, which is 0.98 (95% CI 0.94-1.00).

CONCLUSION

Soluble transferrin receptor value was useful tool for assessment of iron status in patients with CKD, however, they are at best value in combination with the existing conventional tests as serum ferritin and TSAT. Between sTfR and sTfR index, the index has a higher discriminating power. Calculation of both serum sTfR and sTfR index are able to identify pure IDA, ACD and ACD with existing iron deficiency and so providing an alternative non-invasive to bone marrow examination.

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