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Original article

Evaluation of Serum Soluble Transferrin Receptors in Detection of Iron Deficiency Anemia in Chronic Kidney disease Patients on Hemodialysis

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Abstract

Background: A increased risk of morbidity and death is associated with anemia, which is a well-known complication of chronic kidney disease (CKD), particularly in patients with end-stage renal disease who need frequent hemodialysis. Anemia strikes between 49% and 55% of CKD patients, and it becomes more common as the illness progresses. One of the most important signs of iron deficiency in the population-based survey is anemia. **Objective**: In hemodialysis-dependent patients with chronic renal disease, we evaluated the accuracy of serum soluble transferrin receptors for the diagnosis of iron deficiency anemia. **Subjects and methods:** we recruited 90 patients of both sexes with end-stage renal disease, ranging in age from 20 to 60 years. A full blood tests, including those for iron, ferritin, total iron binding capacity, saturation with transferrin, and soluble transferrin receptor, were performed on each patient. The data was analyzed using the Pearson correlation and the independent sample t test. It was

considered significant when the P value was less than 0.05. **Results**: The results of our research showed that mean SsTFR level was (55.14±34.2), ranged between 0.3 and 125.9,there was no statistical significant correlation with p-value >0.05 between SsTFR level and all other iron profile tests (hemoglobin, serum iron, TIBC, ferritin, and transferrin Saturation) and there was no a statistical significant higher level SsTFR with p-value 0.05 in cases on dialysis for more than 6 months, and not treated with Erythropoietin and there was also no statistical significance difference in SsTFR level as regards level of ferritin, or T. saturation.. **Conclusion**: We conclude that serum soluble transferrin receptors is insignificant in detection of iron deficincy anemia in our CKD patients on hemodialysis.

1. Introduction:

The impairment of kidney function that happens gradually over time is called chronic kidney disease (CKD). One well-known side effect of chronic kidney disease (CKD) is anemia, which may be brought on by either a decrease of erythropoietin or an iron shortage from poor absorption or use. For CKD patients to produce red blood cells as optimally as possible, there should be a healthy balance between promoting erythropoiesis and keeping iron levels sufficient.[2]

It is essential to precisely assess the iron status of anemic patients undergoing hemodialysis in order to distinguish between those with functional iron shortage and those with absolute iron deficiency. Because chronic kidney disease (CKD) is a pro-inflammatory condition, there is known to be significant biological variability in serum iron, transferrin saturation, and ferritin levels. This variability complicates the clinical interpretation of these results and can lead to an inaccurate perception of the body's iron status .[3]

Serum soluble transferrin receptor (SsTfR) has 760 amino acids and is necessary for iron to enter cells. The availability of iron in the bone marrow is measured by SsTfR. Erythropoiesis stimulating agents (ESAs) cause the bone marrow to become more activated, which increases the expression of transferrin receptors on the surface of erythroblasts, which are the progenitors of red blood cells .[1]

Serum transferrin (sTfR) is elevated in iron deficiency anemia and severe erythropoiesis and decreases in erythroid hypoplasia and aplastic anemia. When erythropoiesis is restricted due to iron shortage, the erythroblasts' surface releases more sTfR, which raises the level of sTfR in the blood. When diagnosing anemia and tracking the erythropoietic response during anemia therapy, the blood's level of sTfR is a helpful measure .[4]

Since the blood concentration of SsTfR is independent of inflammation, it provides a clinically superior measure of the iron status in inflammation .[5]

2. Patients And Methods:

This cross sectional study was conducted in at internal medicine department at Beni-Suef university hospital. This study was conducted on 90 chronic kidney disease Patients on hemodialysis were divided into sub groups according to their serum ferritin,transferrin saturation,,duration of dialysis and erythropoietin stimulating agents treatment and degree of anemia.

Inclusion criteria

Men and women in the age range of 20 to 60.

Who have been diagnosed with end-stage renal disease (ESRD) and regularly visit the nephrology outpatient department for follow-up will be chosen at random to be involved in the research.

Exclusion criteria: patients who have

- Bleeding.
- Hemolytic anemia.
- Liver disorders.
- Infections
- The use of ascorbic acid and iron supplements orally or intravenously.

Ethical considerations: Providing patients with complete information about the study and the marker .Written informed consent was obtained from all included participants. Approval of the ethical committee of Beni-Suef University was taken. Approval No:FMBSUREC/01112021/Kamal

Methodology

The patients will be subjected to :

1- A thorough medical history should be taken, with particular attention to bleeding, hemolytic anemia, liver disorders, infections, and the use of oral or intravenous iron and ascorbic acid supplements. The patients gave their written permission.

2- Routine laboratory investigation include:

a- CBC

b- Kidney function tests (creatinine, urea)

c- Serum iron

d- TIBC

e- Ferritin

3- Special test.

4- Eliza approach for measuring the serum amount of soluble transferrin receptor.

Reagent preparation

Reagents	Quantity	Reagents	Quantity
Pre-coated, ready to use 96-well strip plate	1	Plate sealer for 96 wells	2
Standard	2	Diluent Buffer	1×45mL
Detection Reagent A	1×120μL	TMB Substrate	1×9mL
Detection Reagent B	1×120μL	Stop Solution	1×6mL
Wash Buffer (30 × concentrate)	1×20mL	Instruction manual	1

First, let everything in the kit, including the samples, cool to ambient temperature (around 18-25 degrees Celsius).

Prior to application.

2. Prepare the Standard by mixing it with 1.0 mL of diluent buffer.

Shake lightly (not to froth) and let sit at room temperature for 10 minutes.

The standard is present at a concentration of 100ng/mL in the stock solution.

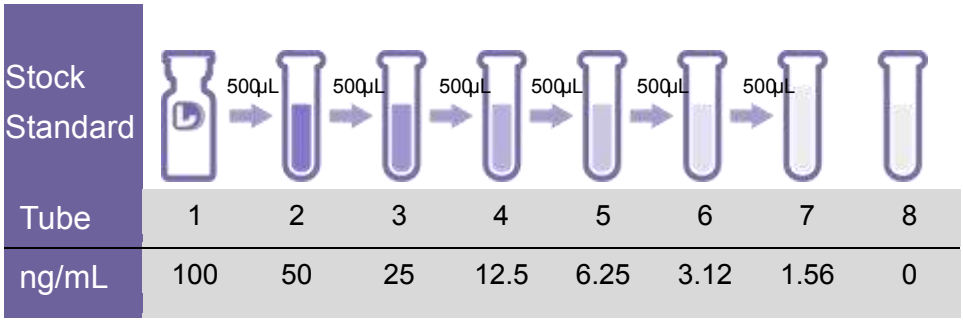
Dilute the sample by adding 0.5 mL of diluent buffer to each of seven test tubes.

Method for making a series of double dilutions in accordance with the illustration

Presented here. Completely combine all tubes prior to the subsequent transfer.

Example: 100 ng/mL in a 7-point dilution series in micrograms per milliliter, 50, 25, 12, 15, 6, 25, 3, 12, 1, 56,

Lastly, the 0ng/mL blank EP tube contains diluent buffer.



3. The stock solutions of Detection A and Detection B should be briefly spun in a centrifuge before use.

Use Diluent Buffer to dilute each component to the appropriate working concentrations. (1:100).

4. Wash Solution - Use 20 milliliters of the concentrated Wash Solution (30 times) and dilute it with 600 milliliters of Wash with 580 milliliters of distilled or deionized water

Solution (1×).

5. Substrate for TMB - Aspirate the solution dose required using

Clean points. Reusing the vial is not an option for the remaining solution.

Statistical Analysis

Data was gathered, coded to enable data manipulation, and then entered into Microsoft Access. The statistical package of social science (SPSS) program, version 22, was used to analyze the data on a Windows 7 computer (SPSS Inc., Chicago, IL, USA).

3. Results:

Table (1): The table showed that the study group's mean age was 45.8±14.4, with a range of 18 to 60 years, and that 42.2 percent of the participants were female and 57.8 percent of the participants were male.

Variables	Number (n=90)	
Age (years)		
Mean ±SD	45.8±14.4	
Median (Range)	51.5 (18-60)	
Sex		
Male	52	57.8%
Female	38	42.2%

Table (2): The table illustrated that the median hemoglobin level was (8.5), median serum iron was (49), for median TIBC it was (215.5), ferritin level show a median of (342), and finally Transferrin saturation the mean was (21.5).

Variables	Median	Range
Hemoglobin	8.5	4-10.5
Serum iron	49	8-620
TIBC	215.5	102-417
Ferritin	342	3-3106
T. saturation	21.5	4-350

Table (3): The table illustrated that the mean SsTFR level was (55.14±34.2), ranged between 0.3 and 125.9.

Parameter	SsTFR
Mean ± SD	55.14±34.2
Median	50.6
Range	0.3-125.9

Table (4): The table illustrated that there was no statistical significant difference with p-value >0.05 between males and females as regards all iron profile tests (hemoglobin, serum iron, TIBC, ferritin, T.Saturation, and SsTFR levels).

Variables	Males (N=52)		Females (N=38)		P-value	Sig.
	Mean	SD	Mean	SD		
Hemoglobin	8.43	0.99	8.38	1.3	0.88	NS
Serum iron	71.56	87.3	49.1	23.1	0.20	NS
TIBC	224.8	66.3	224.03	60.4	0.91	NS
Ferritin	485.9	578.9	545.3	639.6	0.88	NS
T. saturation	28.8	19.5	32.1	55.1	0.27	NS
SsTFR	51.9	32.8	59.5	36.04	0.32	NS

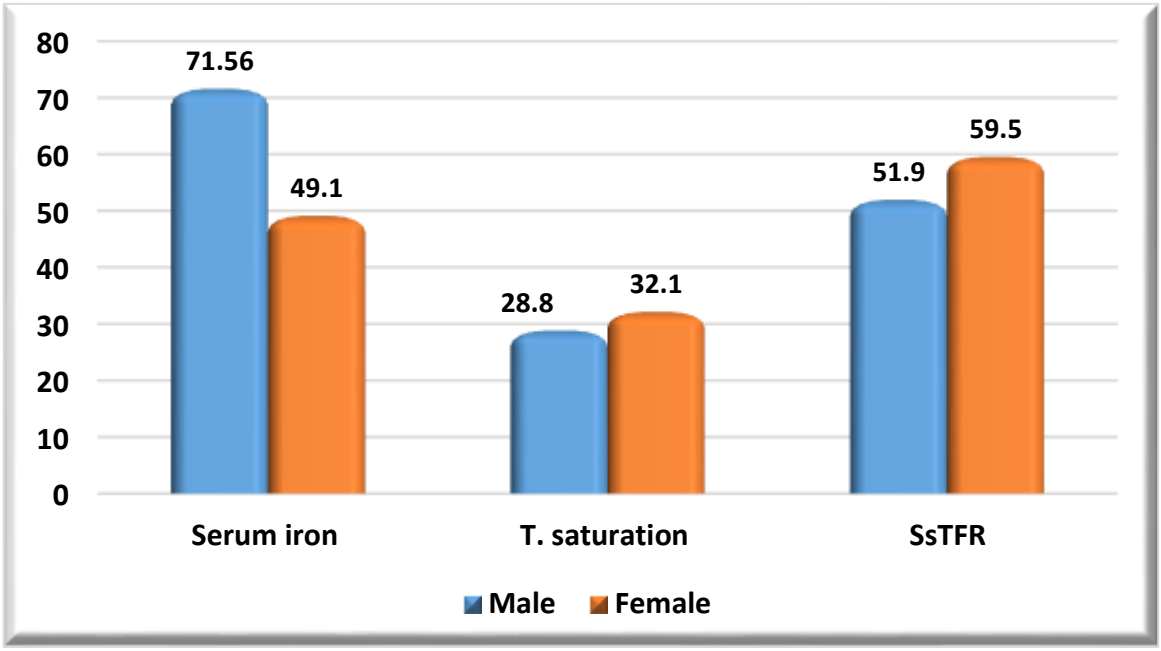


Figure (10): Iron ,TSAT and ssTFR in relation to sex.

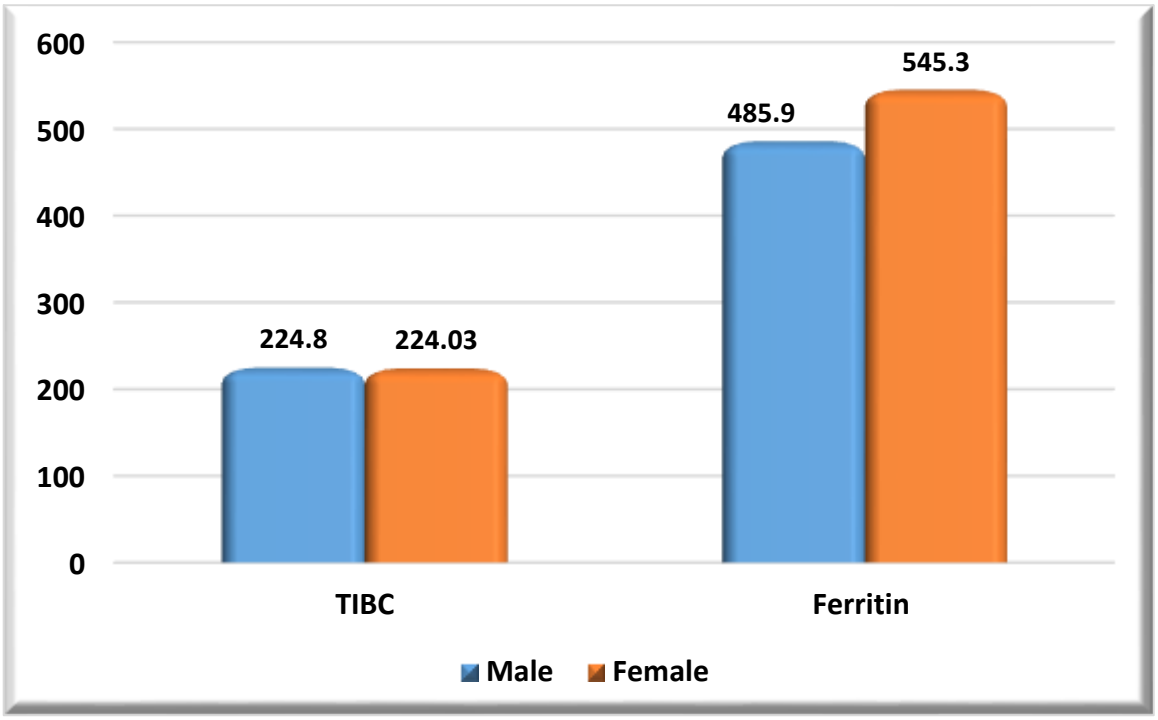


Figure (11): TIBC and Ferritin in relation to sex.

Table (5): The table illustrated that there was no statistical significant correlation with p-value >0.05 between SsTFR level and all other iron profile tests (hemoglobin, serum iron, TIBC, ferritin, and T.Saturation)

Variables	SsTFR level		
	R	P-value	Sig.
Age (years)	0.20	0.06	NS
Hemoglobin	0.07	0.5	NS
Serum iron	-0.009	0.93	NS
TIBC	-0.003	0.97	NS
Ferritin	0.17	0.11	NS
T. saturation	-0.04	0.68	NS

Table (6): The sensitivity and specificity test for iron profile tests illustrated a significant sensitivity to serum iron to differentiate between mild and moderate levels of anemia with a sensitivity of (84.6%) and a specificity of (54.2%) at cutoff (49) with p-value 0.02. on the other hands there was no significant sensitivity to other iron profile tests (TIBC, ferritin, T.Saturation, and SsTFR levels) .

Variable	Sensitivity	Specificity	AUC	Cut off point	P-Value
Serum iron	84.6%	54.2%	70.1%	49	0.02*
TIBC	61.5%	38.9%	51.8%	202.5	0.84
Ferritin	76.9%	47.2%	66%	299	0.07
T. saturation	61.5%	72.2%	64.5%	26.5	0.09
SsTFR	69.2%	50%	51.1%	49.5	0.9

Table (7): The sensitivity and specificity test for iron profile tests illustrated a significant sensitivity to TIBC to differentiate between moderate and sever levels of anemia with a sensitivity of (80%) and a specificity of (72.2%) at cutoff (189.5) with p-value 0.005. on the other hands there was no significant sensitivity to other iron profile tests (serum iron, ferritin, T.Saturation, and SsTFR levels)

Variable	Sensitivity	Specificity	AUC	Cut off point	P-Value
Serum iron	80%	69.4%	63.1%	36.5	0.33
TIBC	80%	72.2%	87.5%	189.5	0.005*
Ferritin	80%	63.9%	59.3%	397.5	0.47
T. saturation	80%	47.2%	59.3%	18.5	0.49
SsTFR	80%	48.6%	52.2%	51.44	0.89

Table (8): The table illustrated that cases on dialysis for more than 6 months were 48.9%, 62.2% of cases had ferritin level >200, 38.9% of them had T-saturation less than 25%, and 48.9% of cases were not treated with Erythropoietin.

Groups	Mean SsTFR level	
	No	%
CKD Patient on regular hemodialysis		
< 6 months	46	51.1%
> 6 months	44	48.9%
Serum ferritin		
<200	34	37.8%
>200	56	62.2%
T.saturation		
<25%	55	61.1%
>25%	35	38.9%
Erythropoietin treatment		
Yes	46	51.1%
No	44	48.9%

Table (9): The table illustrated that there was a statistical significant higher level SsTFR with p-value 0.05 in cases on dialysis for more than 6 months, and not treated with Erythropoietin. On the other hands there was no statistical significance difference in SsTFR level as regards level of ferritin, or T. saturation.

Groups	Mean SsTFR level		P-value	Sig.
	Median	(range)		
CKD Patient on regular hemodialysis				
< 6 months	47.3	0.62-98.8	**0.05**	**S**
> 6 months	65.6	0.30-125.9		
Serum ferritin				
<200	35.7	0.30-125.9	0.11	NS
>200	57.9	4.1-119.7		
T.saturation				
<25%	59.5	0.30-125.9	0.48	NS
>25%	45.2	4.12-119.1		
Erythropoietin treatment				
Yes	47.3	0.62-98.7	**0.05**	**S**
No	65.6	0.30-125.9		

4. Discussion:

A increased risk of morbidity and death is associated with anemia, which is a well-known complication of chronic kidney disease (CKD), particularly in patients with end-stage renal disease who need frequent hemodialysis. Anemia strikes between 49% and 55% of CKD patients, and it becomes more common as the illness progresses. In addition, anemia is a key sign of iron deficiency in the population-based survey.[1]

Absolute iron deficiency and functional iron deficiency were the two main iron abnormalities found in this sample of patients. The differentiation between them is difficult as the definite tool of diagnosis is bone marrow aspiration which is an invasive intervention. [6]

Despite its continued usefulness as a diagnostic tool for IDA in CKD, iron stained bone marrow aspiration (BMA) is too intrusive to be routinely used in clinical practice. [7] Consequently, a more practical, non-invasive, and trustworthy way to assess iron status is required. Soluble transferrin receptor (sTfR) and the TfR-F index, a ratio of sTfR to log ferritin, are two novel markers that have recently emerged as potential indicators for differentiating IDA from others, particularly anemia of chronic disease (ACD). Inflammation and other chronic diseases do not fully impact these two indicators. [8]

The main results of this study were as follows:

The current study revealed that the mean age of studied patients was 45.8±14.4 years old ranged between 20 and 60 years with 57.8% were males and 42.2% were females.

The present study showed that there was no statistically significant difference between males and females as regards all iron profile tests including hemoglobin, serum iron, TIBC,

ferritin, transferrin saturation, and SsTFR levels.

The current study in consistent with Iyawe **et al, (2018)** who revealed that there was no statistically significant difference between males and females in median values of iron indices as regards serum iron, transferrin saturation and serum ferritin.[12]

Our results illustrated that the median SsTFR level was 50µg/ml , ranged from 0.3 to 125.9.

In contrast with our findings, **Suega et al., (2019)** demonstrated that the median soluble transferrin receptor (sTfR) was 0.61 µg/ml, ranged from 0.16 to 4.23 µg/ml.[13]

The current study revealed that there was no statistically significant correlation between SsTFR level and all other iron profile tests including hemoglobin, serum iron, TIBC, ferritin, and transferrin saturation.

The present study in agreement with **Majeed et al., (2016)** who illustrated that there was non-significant negative correlation between sTfR and SF, TSAT and serum iron. While they demonstrated that negative and significant correlation was observed between sTfR levels and Hb and HCT.[10]

In contrast, the present study disagreed with **Gupta et al., (2016)** who revealed that a statistically significant correlation was observed between sTfR and serum iron, TSAT and hemoglobin.[9]

The current study illustrated a significant sensitivity to serum iron to differentiate between moderate and sever levels of anemia with a sensitivity of 84.6% and a specificity of 54.2% at cutoff 49. Also, we found a significant sensitivity to TIBC to differentiate between moderate and sever levels of anemia with a sensitivity of 80% and a specificity of 72.2% at cutoff 189.5.

Also, our results in line with **Gupta et al.,(2016)** who revealed that the cut-off value of TIBC was 272.5 with maximum sensitivity 52.7% and specificity 67.6%.[9]

The present study reported that 51.1% of cases had dialysis for less than 6 months, 62.2% of cases had ferritin level >200, 61.1% of cases had T-saturation less than 25% and 46 (51.1%) cases had erythropoietin treatment. Also, we revealed that there was a statistical significant higher level SsTFR in cases on dialysis for more than 6 months, and not treated with Erythropoietin. On the other hands there was no statistical significance difference in SsTFR level as regards level of ferritin, or T. saturation.

Similarly, our findings in line with **Wande et al., (2023)** who reported that 19 (31.1%) had erythropoietin treatment and 42 (68.9%) patients did not receive erythropoietin treatment.[11]

The current study in line with **Yusra et al., (2022)** who revealed that 107 (84.3%) had erythropoietin treatment and there was no statistically significant differences in the SsTFR levels regarding erythropoietin treatment.[14]

In contrary to our study, researchers in Pakistan found that sTfR could tell the difference between an iron surplus and a deficit in hemodialysis patients. .[10]

According to TARNG et al. (2002), there was a statistically significant negative correlation between sTfR values and SF and TSAT.[15]

Similar to our study **Beerenhout et al., (2015)** noticed that sTfR and SF were not related.[16]

5. Conclusion:

We conclude that serum soluble transferrin receptors is insignificant in detection of iron deficincy anemia in our CKD patients on hemodialysis.

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