

Assessment of Iron Deficiency in Pregnant Women by Using Soluble Transferrin Receptor - Ferritin Index

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ABSTRACT:

BACKGROUND:

Iron deficiency could have an adverse effect on the health of pregnant women and their fetuses. In present, no marker could be considered as the ideal for the detection of Iron deficiency anemia in pregnant women. Some authors suggested that soluble transferrin receptor-ferritin index could be a promising marker for iron deficiency during third trimester of pregnancy.

OBJECTIVE:

To study the value of soluble transferrin receptor - ferritin index as a marker for iron deficiency during third trimester.

PATIENTS AND METHOD:

A cross-sectional study conducted at the Antenatal clinic in Al Zahra'a maternity and children hospital and Baquba Teaching Hospital in Diyala province /Iraq, during the period from 17th march 2016 to the end of March 2017, seventy pregnant women were included in the study. Hemoglobin, CBC, HCT, MCV, and RDW-CV were tested by using automated device. Serum Ferritin and Transferrin receptor was tested by using ELISA kit,

RESULT:

The mean age of the women was 28.3 ± 6.5 (range: 16 – 48) years . Iron deficiency anemia was reported in 32% of the studied group. Serum Iron and Ferritin were significantly lower in iron deficiency anemia group than normal group, ($P < 0.05$). Soluble Transferrin receptor (sTfR) and sTfR- ferritin index were significantly higher in iron deficiency anemia group than the normal group ($P < 0.05$).

CONCLUSION:

S. ferritin is excellent predictor of IDA and had higher sensitivity, specificity and accuracy rates than sTfR and sTfR-F index. Additionally, the validity parameters of sTfR-F index were higher than that of sTfR.

KEY WORDS: IDA, Soluble Transferrin receptor and sTfR-F index.

INTRODUCTION:

Among the elements required by the human body, Iron is the one that plays the most crucial function of them all. It is therefore important for the normal growth and development of a human being. It is used to accomplish some functions in the human body which include; synthesis of the DNA, transportation of oxygen, production of adenosine triphosphate (ATP), protection against oxidation of the cell components, and production of mitochondria^(1, 2).

Iron in the body of a human being is stored as hemosiderin in the spleen, duodenum, marrow; liver, skeletal muscle including the remaining anatomic areas, as well as in the form of ferritin.

The latter acts as the reserve for iron and is the main protein storage⁽³⁾. World Health Organization (WHO), the percentage of women who are anemic is 52 % for those who are pregnant and approximately 42% of the whole women population. This statistic applies to the women population in the developing countries. The con

dition for anemia is major as a result of a deficiency in iron⁽⁴⁻⁷⁾. During the third trimester of pregnancy, the requirement for iron is greatly increased which is usually higher as compared to the other trimesters standing at 840 mg. The increase in the requirement for iron during pregnancy is attributed to the need to produce certain enzymes, synthesis of the hemoglobin, and to be used for the development of the fetus⁽⁸⁻¹⁰⁾.

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AIM OF THE STUDY:

To study the value of soluble transferrin receptor - ferritin index as a marker for iron deficiency during third trimester of pregnancy.

PATIENTS AND METHOD:

Study design, setting and time:

A cross-sectional study conducted at the Antenatal clinic in Al Zahra'a maternity hospital and Baquba Teaching Hospital in Diyala province Middle East of Iraq during the period from 17th march 2016 to the end of March 2017.

PATIENTS:

The study included seventy five pregnant women aged 15-45 years in their third trimester (from 28 weeks till delivery). Women who met the inclusion criteria were recruited from antenatal clinic in Al Zahra'a maternity and children hospital and Baquba Teaching Hospital in Diyala province. The subjects were asked to participate in the study; verbal and signed consent was obtained from each participant prior to participation or recruited in the study.

2.3. Exclusion criteria

Women with one or more of the following conditions were excluded from the study:

1. Women diagnosed as having any type of anemia other than IDA.
2. Having chronic or inflammatory diseases or currently using certain medications which tend to alter iron levels.
3. History of current bleeding, previous abortion, sever infection, trauma, preeclampsia.
4. Current or recent Iron therapy or blood transfusion

2.4. Blood Samples Collections:

A 5 ml of venous blood was drawn from each participant woman and sent to the lab for complete blood count, peripheral smear examination, the HGB, HCT, MCV and RDW-CV, WBC count and differentiation were tested using automated method. Serum Ferritin and Transferrin receptor were estimated by specially ELISA kit each one.

The samples of 5 ml venous blood were collected under aseptic clean technique Two ml of peripheral blood were put into EDTA tubes for complete blood count and peripheral blood smear. The remaining three ml of blood was put into an iron free plain plastic tube. Serum was separated and stored in different aliquots at - 20°C for Serum Iron, Ferritin and Transferrin Receptor assay.

Serum ferritin

Principle

The test uses a sandwich immune detection method; the detector recombinant protein in buffer binds to antibody in sample, forming recombinant protein-antibody complexes, and migrates onto nitrocellulose matrix to be captured by the other immobilized antigen on test strip. The more antibodies in sample forms the more recombinant protein-antibody complex and leads to stronger intensity off fluorescence signal on detector recombinant protein, which is processed by Instrument for AFIAS-6 to show ferritin concentration in sample

This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Transferrin receptor (TFR). Add Transferrin receptor (TFR) to wells that are pre-coated with Transferrin receptor(TFR)f monoclonal antibody and then incubate. After incubation, add anti TFR antibodies labeled with biotin to unite with streptavidin-HRP, which forms the immune complex. Remove unbound enzymes after incubation and washing, then add substrate A and B. The solution will turn blue and change to yellow with the effect off acid. The shades off solution and the concentration off Human Transferrin receptor (TFR) are positively correlated

The hematological parameters: Hemoglobin, HCT, MCV, and RDW-CV were tested using automated method. Blood samples collected in K2-EDTA tube then the blood is well mixed (though not shaken) and placed on a rack in the automated analyzer NIHON Abbott ® that use photometric pathway for obtaining the results. All procedures with this instrument are automated, and not needed further specimen preparation Results regarding Total WBC and differential counts, RBCs, hemoglobin, HCT, MCVd, MCHd, MCHCd, RDWd, platelets count, PCTd, MPVd, and PDWd, all were printed out.

Data entry and statistical analyses were performed using SPSS (Statistical Package for Social Sciences) software package version 24, the variables were analyzed using descriptive statistics.

RESULTS:

The study included 75 pregnant women at different gestational age (range 28 – 40 weeks), the mean age of the women was 28.3 ± 6.5

TRANSFERRIN RECEPTOR - FERRITIN INDEX

(range: 16 – 48) years (**Table 1**). Iron deficiency anemia was diagnosed by low Hb, PCV, MCV, MCH, MCHC, RBC count as well as low S. Ferritin, S. Iron & sTfR.

24 women giving a proportion of IDA among the studied group of (32%), (Fig. 1), the remaining 51 women (68%) had normal hematological and blood film findings.

Table 1: Demographic characteristics of the studied group.

Variable		No.	%
Age (years)	Mean±SD	28.3±6.5	-
	Range	16-48	-
Gravidity	Gravida 1	22	29.3
	Gravida 2	20	26.721
	Gravida 3	16	21.3
	Gravida 4 or more	17	22.7
Abortion	Yes	21	28.0
	No	54	72.0
Parity	Nulliparous	32	42.7
	One	20	26.7
	Two	8	10.7
	Three or more	15	20.0

SD: standard deviation

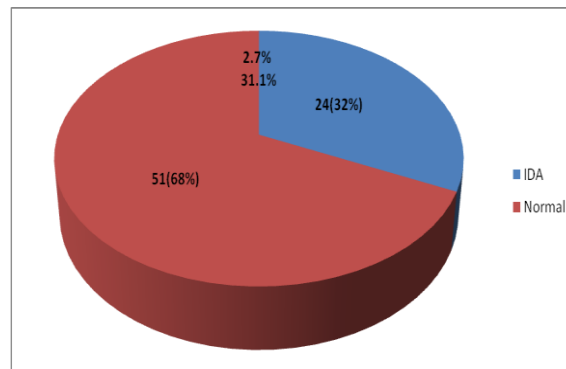


Figure 1: Distribution of the studied group according to the iron status.

Levels of S. Iron and ferritin were significantly lower in IDA than normal group of pregnant women, whereas Soluble Transferrin receptor (sTfR) was significantly higher in IDA group

than normal pregnant women. Similarly, the mean sTfR-F index of IDA group was 8.07 which was significantly higher than the 1.10 in normal group, ($P < 0.001$)(Table 2).

Table 2: Comparison of mean levels of S. Ferritin, Iron, Transferrin receptor and sTfR- ferritin index of IDA and normal women group (N = 75).

Parameter	IDA (n=24)		Normal (n= 51)		P. value
	Mean	SE	Mean	SE	
S. Iron (µg/dL)	7.76	0.68	12.27	1.10	0.009*
S. Ferritin(µg/l)	9.42	2.24	23.68	4.76	< 0.001*
sTfR (mg/l)	7.53	1.07	1.52	0.34	< 0.001*
sTfR-F index (mg/l)	8.07	0.94	1.10	0.26	< 0.001* *
Significant difference at P< 0.05					

SE: standard error of mean

S ferritin had higher sensitivity (87.6%) than sTfR (79.2%) and sTfR-F index (80.2%), however, the differences were statistically insignificant, (P>0.05). The specificity of S ferritin was 91.7%, and it was significantly higher than that of sTfR (72.5%) and sTfR-F index (76.5%), (P = 0.001).

Similarly, the accuracy of S ferritin was higher than that in both sTfR (75.9%) and sTfR-F index (78.4%), (P = 0.025). Also, the positive predictive value (PPV) was significantly higher in S. ferritin (91.3%) than sTfR (74.2%) and sTfR-F index (77.3%), (P = 0.001) (**Table 3**).

Table 3: Validity parameters of S. Ferritin, Iron, Transferrin receptor and sTfR- ferritin index in prediction of IDA.

	S. Ferritin	sTfR	sTfR-F index	P.value
Sensitivity	87.6%	79.2%	80.20%	0.23
Specificity	91.7%	72.5%	76.5%	0.001*
Accuracy	89.7%	75.9%	78.4%	0.025*
PPV	91.3%	74.2%	77.3%	0.001*
NPV	88.1%	77.6%	79.4%	0.092
AUC	0.913	0.842	0.825	0.12
*Significant difference at P< 0.05				

AUC: Area under the curve (ROC)

DISCUSSION:

The present study found that 32% of the studied group had IDA, this was expected as the iron stores reduced with advanced pregnancy particularly at third trimester, the rate of IDA was close to that reported in previous Iraqi studies; Al-Shawi et al. found that 28.4% of pregnant women at the third trimester in Baghdad were anemic ⁽¹¹⁾ Another Iraqi study was conducted by Ahmad HM in Khaki camp in Duhok city reported higher proportion of IDA among emigrated pregnant women ⁽¹²⁾. From other point of view, Gatea et al. (188) reported that 58 women (22.4%) were in the third trimester of them 46 had IDA and higher proportion of them with mild to moderate IDA ⁽¹³⁾. Al-Mehaisen et al ⁽¹⁴⁾ found that 42.5% of pregnant women at third trimester had IDA. In Saudi Arabia, Apatan et al. found IDA in 58% of pregnant women ⁽¹⁵⁾, In Pakistan IDA documented in 46% of pregnant women at 3rd trimester ⁽¹⁶⁾, in India a prevalence

of 69.5% was reported by Abel et al. in 2001 ⁽¹⁷⁾, this higher results may be due to different dietary habits among countries.

The current study found that the mean Serum Iron and mean S. Ferritin were significantly lowered in IDA group than that of normal group. Similar to the study done in Pakistan, by Raza et al. ⁽¹⁸⁾ who found significant decreased in S. iron and S. ferritin levels particularly in third trimester compared to first trimester and controls.

The present study found that sTfR and sTfR-F index were significantly higher in IDA group, Madhavan et al. ⁽¹⁹⁾ found that sTfR increased significantly with increased gestational age to reach its peak level and almost doubled at the third trimester compared to its levels in the first trimester and in non-pregnant women, additionally, authors suggested that sTFR can be used as an indicator for IDA in pregnant women ⁽¹⁹⁾. validity of sTfR and sTfR-F index in

prediction of IDA was good in term of sensitivity specificity and accuracy the sTfR had a sensitivity of 79.2%, Specificity 72.5% and accuracy of 75.9%, the corresponding rates for the sTfR-F index were 80.2%, 76.5% and 78.4%.

Previous studies assess the role off sTfR and sTfR-F index to identify IDA in different diseases a study conducted by Oustamanolakis et al.(Greece 2011) ⁽²⁰⁾ suggested that sTfR-F index seems to be very efficient in the detection and diagnosis off IDA and that sTfR-F index validity was better than that off sTfR moreover the Greek study found that the sensitivity off high sTfR levels for diagnosis IDA was 81%, specificity was 80%, whereas positive predictive value was 63% and NPV was 91%. The sensitivity off high sTfR-F index for diagnosis IDA was 91% and the specificity was 92%, whereas positive predictive value was 83% and NPV was 96%, and both tests were comparable to S. ferritin, moreover a study was conducted by Infusion et al. ⁽²¹⁾ reported that sTfR was more efficient than sTfR-F index This is a relevant conclusion, because previous studies suggested that sTfR index may increase diagnostic precision providing higher sensitivity and specificity than sTfR determination alone ^(21, 22). However Broek et al. ⁽²³⁾ found that sTfR had a sensitivity of 76.1%, specificity of 45.5%, and accuracy of 60%, which were lower than the validity rates reported in this study. Study was conducted by Akinsooto et al. ⁽²⁴⁾ found that the sensitivity, specificity and accuracy of sTfR were 75%, 63% and 64%, respectively.

CONCLUSION:

Iron deficiency anemia was frequent among sample of Iraqi pregnant women at third trimester, showing lower prevalence rates compared to the national records and neighborhood countries.

Moreover, Serum ferritin showed higher sensitivity, specificity and accuracy in detection of IDA than soluble transferrin receptor and Soluble Transferrin Receptor/ferritin index.

REFERENCES:

1. Atamna H., Walter P.B. and Ames B.N, The Role of Heme and Iron- Sulfur Clusters in Mitochondrial Biogenesis, Maintenance, and Decay with Age. *Archives of Biochemistry and Biophysics.*, 2002; 397: 345-53.
2. McCann J, Ames B.N. An Overview of Evidence for A causal Relation between Iron Deficiency During Development and Deficits in Cognitive or Behavioral function. *American Journal of Clinical Nutrition.*, 2007; 85: 931-45.
3. Saito H. Metabolism of iron stores. *Nagoya J Med Sci.* 2014;76:235–54.
4. Muller, O. and M. Krawinkel. "Malnutrition and health in developing countries." *CMAJ* 2005; 173: 279-86.
5. Scrimshaw NS, Iron deficiency. *Scientific American* 2002; 265: 46-52.
6. School TO. Iron status during pregnancy : setting the stage for mother and iron deficinecy in Women. *Am J Clincial Nutr.* 2005;81:1218–22.
7. Zimmermann, M. B. and R. F. Hurrell (2007). "Nutritional iron deficiency." *Lancet* 370(9586): 511-20.
8. Raza N, Sarwar I, Munazza B, Ayub M, Suleman M. Assessment of iron deficiency in pregnant women by determining iron status. 2011;23:36–40.
9. Soma-Pillay P, Nelson-Piercy C, Tolppanen H, Mebazaa A. Physiological changes in pregnancy. *Hear Dis Pregnancy.* 2007;27:89–94.
10. Khalafallah AA, Dennis AE. Iron deficiency anaemia in pregnancy and postpartum: Pathophysiology and effect of oral versus intravenous iron therapy. *J Pregnancy.* 2012.
11. AL-Shawi ARJ, Obaid JA, Mohammad MR, Mohammed NH. Study the Incidence and Types of Anemia in Pregnant Women in Baghdad Province . 2012;6:2–5.
12. Ahmad HM. Anemia and Iron Deficiency Anemia Prevalence with Serum Zinc assessment among Emigrated Yazidis People in Khanki camp in Duhok city, Kurdistan Region, Iraq. *IOSR J Dent Med Sci.* 2016;15:105–10.
13. Gatea AA, Tawfeeq WF, Hassan MR. The prevalence of iron deficiency anemia in pregnant Women in Ibn- Albaldy Hospital. *Iraqi National Journal of Nursing Speciality.* 2013;26:71–79
14. Al-Mehaisen L, Khader Y, Al-Kuran O, Abu Issa F, Amarin Z. Maternal anemia in rural Jordan: Room for improvement. *Anemia.* 2011; 3: 181-87.

15. Apatan EJ, AlHassan AE, Al-Shammary RA. Iron Deficiency Anemia among Pregnant Women in Hail , Kingdom of Saudi Arabia. IOSR J Nurs Heal Sci Ver I 2015;4:2320–940.
16. Seemal Vehra EMAQ and FA. Effect of Socio- Demographic and Gestational Status on the development of Iron Deficiency Anemia in Pregnant Women. Pakistan J Nutr. 2012;11:643–47.
17. Abel R, Rajaratnam J, Gnanasekaran VJ, Jayaraman P. Prevalence of anaemia and iron deficiency in three trimesters in Rural Vellore district, South India. Trop Doct. 2001;31:86–89.
18. Raza N, Sarwar I, Munazza B, Ayub M, Suleman M. Assessment of iron deficiency in pregnant women by determining iron status. J Ayub Med Coll. 2011;23:36–40.
19. Madhavan Nair K, Bhaskaram P, Balakrishna N, Ravinder P, Sesikeran B. Response of hemoglobin, serum ferritin, and serum transferrin receptor during iron supplementation in pregnancy: A prospective study. Nutrition. 2004;20:896–99.
20. Oustamanolakisa P, Koutroubakisa IE, Messaritakisb I, Ninirakic M, Kouroumalisa EA. Soluble transferrin receptor-ferritin index in the evaluation of anemia in inflammatory bowel disease: A case-control study. Ann Gastroenterol. 2011;24:108–14.
21. Infusino I, Braga F, Dolci A, Panteghini M. Soluble transferrin receptor (sTfR) and sTfR/log ferritin index for the diagnosis of iron-deficiency anemia: A meta-analysis. Am J Clin Pathol. 2012;138:642–49.
22. Koulaouzidis A, Said E, Cottier R, Saeed AA. Soluble transferrin receptors and iron deficiency, a step beyond ferritin. A systematic review. J Gastrointest Liver Dis. 2009;18:345–52.
23. Broek NR, Letsky EA, White SA, Shenkin A. Iron status in pregnant women: Which measurements are valid? Br J Haematol. 1998;103:817–24.
24. Akinsooto, PJ Ojwang, T. Govender, J. Moodley, CA Connolly V. Soluble transferrin receptors in anaemia of pregnancy. Journal of Obstetrics and Gynaecology. 2001;21:250-52.