Original Article

Soluble Transferrin Receptor as a Marker in The Diagnosis of Iron Deficiency Anaemia in Pregnancy : A Study in Calabar, Nigeria

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Assessing iron status during pregnancy is fraught with difficulties because the profound hemodynamic changes associated with pregnancy affect several indexes of iron status. Current markers of iron deficiency tend to be less reliable in pregnancy especially ferritin which is an acute phase reactant and as such will not be reliable in the diagnosis of iron deficiency anaemia among pregnant women with infections. Soluble transferrin receptor assay may be useful in these situations because it reflects the degree of iron requirement in relation to supply and it is not an acute phase reactant. Our aim was to evaluate the usefulness of soluble serum transferrin receptor (sTfR) in relation to serum ferritn in the diagnosis of iron deficiency and iron deficiency anaemia during pregnancy. Serum iron and soluble transferrin receptor concentration were determined using ELISA technique while haemoglobin concentration was determined using automatic cell counter PCE-210 version 5.10 by ERMA INC. Tokyo. One hundred and fifty consenting pregnant women within the age range of 15-45 years were recruited for the study. In 81.81% of the samples analysed serum ferritin and soluble transferrin receptor agreed on the presence/absence of iron deficiency anaemia.18.18% of the pregnant women that were shown to be without iron deficiency anaemia with serum ferritin (serum iron < 12ng/ml) were shown to have iron deficiency anaemia with soluble transferrin receptor (sTfR> 2.4 ug/ml). The specificity of sTfR was 100%. The sensitivity of sTfRin relation to both anaemia and depleted iron stores was 67.99%, but this figure may not be a true reflection of sensitivity because of small sample size. sTfR during the first trimester was low (1.34±0.48) but increased significantly (p < 0.05) in second (2.98±0.72) and third trimester (2.59±0.73). The prevalence of iron deficiency anaemia was shown to be 18.0% when using serum ferritin and haemoglobin as markers (SI < 12ng/ml and Hb< 11g/dl) and 22.0% when soluble transferrin receptor and haemoglobin were used as markers (sTfR> 2.4ug/ml and Hb< 11g/dl) and the difference was statistically significant (p < 0.5).Conclusions: sTfR seems to be a more specific and sensitive marker of iron deficiency anaemia in pregnancy when compared to serum ferritin especially in the presence of infection.

Key words: Anaemia, Soluble Transferrin Receptor, Ferritin, Pregnancy

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Introduction

Assessing iron status during pregnancy can be difficult because during pregnancy hemodilution leads to a reduced haemoglobin concentration, whereas both serum iron and ferritin concentrations decrease and total iron-binding capacity increases[1]. However relative contribution of pregnancy per se and a pregnancy induced negative iron balance in bringing about these changes can be assessed by measuring the changes in hemoglobin, serum iron, serum ferritin, and total iron-binding capacity that occur during pregnancy[1,2]. The absence of iron stores (iron deficiency) can be diagnosed by showing that there is no stainable iron in the reticulo-endothelial cells in bone marrow smears. Bone marrow examination is generally regarded as the definitive marker of iron deficiency. Such examination is uncomfortable, cumbersome and impractical for routine use. There is a clinical need for non-invasive and sensitive means of detecting iron deficiency and a possible approach is the estimation of serum ferritn (SF) and of recent, serum soluble transferrin receptor (STfR).

Iron stores in the body exist primarily in the form of ferritin, with small amount found in the plasma.The concentration of this serum ferritin is positively correlatedwith the size of the total body iron stores in the absence of inflammation. Alow serum ferritin value reflects depleted iron stores, but not necessarilythe severity of the depletion. In pregnancy, serum ferritin concentration is maximum at 12–16 weeks gestation, then falls with advancing gestation to reach a nadir at the third trimester[3,4,5].The use of serum ferritin has improved the diagnostic accuracy of iron deficiency. Its practical value is somewhat reduced, however, by the fact that serum ferritin is a very sensitive acute-phase reactant and may be increased for weeks after an infection with fever for a day or two[6,7]. Several other conditions, such as use of alcohol[7], liver disease, and collagen diseases, may also increase serum ferritin concentrations.

Soluble transferrin receptor (sTfR) is present in human plasma and is considered as a truncated form of the tissue receptor that exists as a transferrin-receptor complex and therefore it reflects tissue iron deficiency[8,9]. The plasma transferrin receptor concentration is not increased with infection or inflammation, unlike plasma ferritin. Hence, measurement of the plasma transferrin receptor concentration may be especially helpful in the task of differentiating between anaemia of iron deficiency and the anaemia associated with chronic inflammatory disorders. Determination of transferrin receptors in plasma has also been recommended in the diagnosis of iron deficiency. Its advantage is that it is not influenced by infections[10].

The use of a combination of determinations of serum ferritin and serum transferrin receptors has also been suggested[11]. The serum ferritin level varies with iron stores, while STfR is assumed to reflect the degree of tissue iron supply[12]. The two major determinants of the level of soluble transferrin receptor are body iron status and the bone marrow erythroid expansion and activity[13]. Cytokines such as tumour necrosis factor-α and interleukin-6 have been suggested to reduce transferrin receptor expression in in-vitro experiments[10]. In anaemia of chronic disease, soluble transferrin receptor level is not affected by the reduced plasma iron concentration, since there is a concurrent increase in the cytokine levels. In a study by Skikne*et al,*[14]results indicated that serum ferritin is the most sensitive index of iron status when there are residual iron stores, whereas the serum soluble transferrin receptor is more sensitive when there is functional iron deficiency. This study is aimed at evaluating the use of soluble transferrin receptor and haemoglobin as markers in the diagnosis of iron deficiency anaemia in pregnancy compared to serum ferritin and haemoglobin.

Materials and Method

One hundred and fifty pregnant women within the ages of 15-45 years were used as subjects in this study; fifty of the pregnant women were from antenatal clinic of University of Calabar Teaching Hospital,Calabar, fifty were from St. Joseph's Hospital, Ikot Ene in Akpabuyo Local Government Area and the remaining fifty pregnant women were from University of Calabar Teaching Hospital extension clinic in Okoyong, Odukpani Local Government Area, all in Cross River state, Nigeria. The subjects were given questionnaires to fill which provided useful information for this study. All the participating pregnant women signed a consent form before being recruited into the study and ethical clearance for the study was also obtained from Cross River State Ministry of Health, Calabar, Nigeria.

Haemoglobin concentration was measured using full automatic blood cell counter, PCE-210 version 5.10 by ERMA INC. Tokyo.Ferritin was determined using human ferritin enzyme immunoassay test kit by Diagnostic Automation, Inc. Calabasas USA.Soluble Transferrin Receptor was measured using human soluble

transferrin receptor ELISA Kit by Biovendor Diagnostics USA. The manufacturer's instructions were followed when using all the kits.

All statistical analyses were performed using the program Statistical Package for Social Sciences (SPSS) for windows version 16.0, SPSS Inc., Chicago, US. One-way analysis of variance (ANOVA) and Chi-square were used.Sensitivity was defined as TP/TP + FN x 100 and specificityas TN/TN + FP x 100, where TP is true positive, FN is false negative,TN is true negative, and FP is false positive. Positive predictivevalue was defined as TP/TP + FP x 100 and negativepredictive value as TN/TN + FN x 100.

RESULTS

Demographic characteristics of the pregnant women are presented in table 1. The mean age of pregnant women that participated in this study was shown to be 29.0 ±11.0, 106(70.67%) of women were within the age range of 15-30 years while 44(29.33%) of women were within the age range of 31- 45 years of age. The table shows that significantly (P<0.05) higher percentage of pregnant women79(52.67%) had secondary education when compared to those with primary, 33 (22.00%), and tertiary education 38(25.33%). Forty six (46.66%) of the pregnant womeninvolved in this study were on oral iron supplementation. The table also shows that significantly higher (P< 0.05) percentage of pregnant women involved in this study were not employed92 (61.33%) while the remaining 58(38.67%) were gainfully employed.Prevalence of iron deficiency anaemia among the pregnant women was assessed independently using serum ferritin (<12ng/ml) and soluble transferrin receptor (> 2.4ug/ml) in relation to haemoglobin (11g/dl).The result is presented on table 2. The result shows that soluble transferrin receptor detected a higher incidence33 (22.0%) of iron deficiency anaemia that is statistically significant (P < 0.05) when compared to serum ferritin 27(18.00%). The table also shows that 18.18% of the women that were without iron deficiency anaemia with serum ferritin were shown to have iron deficiency anaemia with soluble transferrin receptor.

Table 1 Demographic characteristics of the study group

Variables	*Number of pregnant women (150)*
Mean Age (yrs)	*29±11.0*
15yrs – 30yrs	*44(29.33)*
31yrs – 45yrs	*106(70.67)*
Level of education	
Primary	*33(22.00)*
Secondary	*79(52.67)***
Tertiary	*38(25.33)*
Iron supplementation	
Yes	*70(46.66)*
No	*80(53.33)****
Employment	
No	*92 (61.33)**
Yes	*58(38.67)*

Values are given as number (%).

** means significantly higher (p <0.05) when compared to those with secondary and tertiary education

* means significantly higher (p < 0.05) when compared to those that were employed.

*** means significantly higher (p < 0.05) when compared to those that were on iron supplementation.

Table 2 Prevalence of Iron Deficiency Anaemia using SF and sTfR as Markers

Serum ferritin (SF) n=150	*27(18.00%) **
Soluble transferrin receptor (sTfR) n=150	*33(22.00)**
*% of women that were without IDA using SF6(18.18%)** but had IDA with sTfR (n=33)*	
% of women that had IDA with both SF and sTfR (n=33)	*27(81.81)***

**The difference is statistically significant when compared to soluble transferrin receptor (P< 0.05).*
*** The difference is statistically significant (P< 0.05).*
IDA means Iron deficiency anaemia

The specificity, sensitivity, positive predictive value, and negative predictive value of sTfR in relation to iron deficiency status as defined by serum ferritin and haemoglobin are shown in Table 3.The table showed the specificity of sTfR to be 100% while sensitivity was 67.99%. The positive predictive value was shown to be 100% while negative predictive value was 89.17%. When the markers were evaluated in relation to gestational age, the pregnant women that were within the first trimester recorded the lowest concentration of soluble transferrin receptor and haemoglobin while serum ferritin and soluble transferrin receptor were significantly higher(P< 0.05) in the second trimester (Table 4). The table shows the concentration of SF(ng/ml), sTfR(ug/dl) and Hb(g/dl) in the first trimester to be 31.09±5.10, 1.34±0.48 and 10.89 ± 0.27 respectively, the concentration of the parameters for the second trimester were shown on the table to be 43.75±6.61, 2.98±0.72 and 11.09±0.21 respectively, that of the third trimester were shown to be 29.90±4.14, 2.59±0.73, 11.11± 0.20 respectively. Percentage of women that had abnormal value of soluble transferrin receptor and serum ferritin were found to increase steadily from first to third trimester. The table showed the percentage of women with abnormal value of SF, sTfR and Hb to be 34.29%, 20.00% and 51.43% for the first trimester, 35.29%, 21.57%, and 52.94% for the second trimester, and 35.94%, 23.43% and 48.44% for the third trimester. Haemoglobin concentration correlated negatively with soluble transferrin receptor (P< 0.05) as shown in the graph.

Table 3 Specificity, sensitivity, negative predictive value (PV-), and positive predictive value (PV+) of soluble serum transferrin receptor (sTfR)

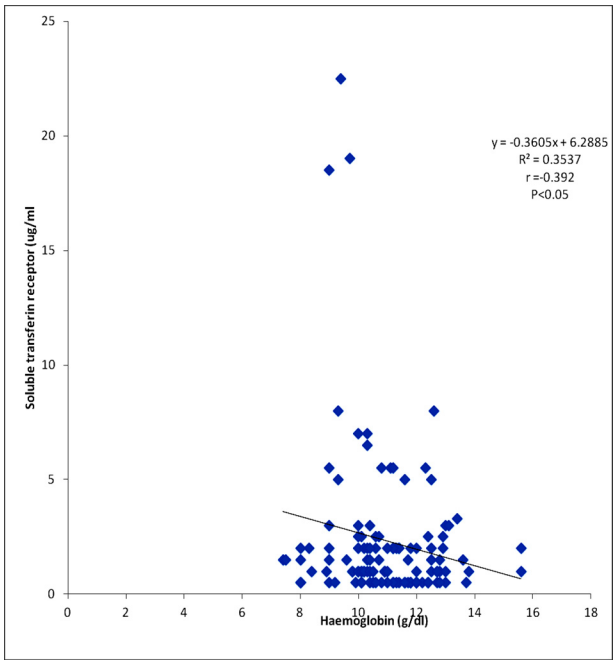
	No. with iron deficiency anaemia (n=27)	No. without iron deficiency anaemia (n=120)
Sensitivity	69.77%	-
Specificity	-	100%
PV-	-	89.17%
PV+	100%	-

Calculations were based on absence of iron deficiency (serum ferritin >12 ng/ml and hemoglobin>11 g/dl) or the presence of iron deficiency (serum ferritin <12 ng/ml and hemoglobin<11 g/dl). Specificity is the fraction of women without iron deficiency that sTfR predicts correctly, sensitivity is the fraction of women with iron deficiency that sTfR predicts correctly, PV- is the fraction of negative results according to sTfR that are true negatives, and PV+ is the fraction of positive results according to sTfR that are true positives

Table 4 Concentrations of serum ferritin, soluble transferrin receptor (sTfR), and hemoglobin (Hb) in different trimesters

First trimester (n=35)	Second trimester (n=51)	Third trimester (n=64)
SF(ng/ml)31.09±5.10*(34.29%)	43.75±6.61* (35.29%)	29.90±4.14* (35.94%)
sTfR(ug/ml)1.34±0.48*(20.00%)	2.98±0.72* (21.57%)	2.59±0.73* (23.43%)
Hb (g/dl)10.89±0.27(51.43%)	11.09±0.21 (52.94%)	11.11±0.20 (48.44%)

The values in bracket are percentage of pregnant women that have abnormal values of SF, sTfR and Hb in different trimesters.
[1]The abnormal value was defined as serum ferritin, <12 ng/ml; sTfR, > 2.4ug/ml and Hb, < 11 g/dl.
*The differences in values are statistically significant (p < 0.05).
The values of SF, sTfR and Hb are presented in Mean ±SEM



Correlation between haemoglobin and soluble transferrin receptor

DISCUSSION

This study was undertaken to evaluate the use of serum soluble transferrin receptor (sTfR) as a marker in the diagnosis of iron deficiency anaemia in pregnancy compared to serum ferritin and haemoglobin. Serum ferritin is particularly useful in diagnosis of iron deficiency anaemia because a level below 12 ng/ml is diagnostic, but the measurement is also influenced by inflammation and is not useful for detecting iron deficiency of recent onset[12]. A small amount of sTfR circulates normally in plasma. It originates from the extracellular chain of transferrin receptor present in the membrane of every cell. The erythroid precursors in the bone marrow are the major determinants of these serum concentrations[15,16]. In conditions with increased erythroid marrow mass there is an elevation in transferrin receptor concentration, whereas in cases of erythroid hypoplasia or aplasia, serum transferrin receptor concentration falls[15,16,17,18]. The concentration of serum transferrin receptor also depends on the adequacy of iron availability to tissues[15,16,17,18,19]. When iron stores are exhausted and iron tissue availability is compromised, an early and progressive rise in serum transferrin receptor concentration occurs[14].

Our results corroborate the view that sTfR is an indicator of iron status in pregnancy. As the age of the pregnancy progresses, there is a gradual increase in mean sTfR concentration. Furthermore, we observed significant (P<0.05) negative correlation between sTfR and haemoglobin concentration. Kling*et al.*[20] described a significant association of sTfR with hemoglobin, transferrin saturation, and serum ferritin. Virtanen *et al.*[21] also observed that sTfR correlates with serum ferritin and mean cell volume in infants.

In this study, the accuracy of sTfR was evaluated in relation to both serum ferritin and hemoglobin, indicating either the absence or presence of iron deficiency. The sensitivity of sTfR was estimated to be 69.77% and specificity 100% unfortunately this result may be less reliable because of the limited number of samples. The sensitivity of sTfR in the present study agrees with the findings in adults of 69% to 94%[10,22,23,24]. In a study in infants carried out by Olivares *etal.,*[23]sTfR showed a low sensitivity (23.6%) and good specificity (98.3%). However, its sensitivity improved when a sTfR> 10 mg/L was selected as the cutoff (sensitivity: 66.5%; specificity: 71.3%). Our findings also shows sTfR to have negative predictive value of 89.17% and a positive predictive value of 100%, this agrees with the result obtained by Akesson*et al.,*[10]who had 99% and 100% respectively. Our result also shows that 18.18% of the women who were shown to be free from iron deficiency anaemia with serum ferritin tested positive with soluble transferrin receptor. The discrepancy in the result may be attributed to the presence of underlying infections like malaria parasite (Nigeria being an endemic area) which made serum ferritin to appear normal even though the women had iron deficiency anaemia. Therefore, it cannot be ruled out that serum ferritin was falsely elevated in these women because serum ferritin can be raised in inflammation[25,26,27,28]. This may explain the high specificity obtained for sTfR receptor in this study and further emphasizes that sTfR may be a better marker for iron deficiency in pregnancy even though no firm conclusions can be drawn regarding the sensitivity of sTfR in this study. Nevertheless, the findings indicate a good accuracy of sTfR compared to serum ferritin in diagnosis of iron deficiency anaemia in pregnancy and support observations from earlier studies that sTfR is not elevated above the reference interval by hormonal alterations in pregnancy or by receptors of placental origin[29, 30]. In conclusion, the sTfR may be a better marker in diagnosis of iron deficiency anaemia in pregnancy especially in malaria endemic areas.

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