Serum transferrin receptor concentrations in women with mild malnutrition\(^1\)–\(^3\)

Solo Kuvibidila, Raj P Warrier, David Ode, and Lolie Yu

**ABSTRACT**  We determined the influence of undernutrition on blood soluble transferrin receptor (sTfR) concentrations, an indicator of iron deficiency, in 99 Zairean women (aged 16–45 y) without inflammation. They were recruited during a survey on iron deficiency in rural Bas-Zaire. sTfR was measured by enzyme immunonassay, and indicators of nutritional status [albumin, transthyretin (or prealbumin), and retinol binding protein] were measured by radial immunodiffusion. Undernutrition was diagnosed if the concentration of any one of the indicators was below normal: albumin < 35 g/L, transthyretin < 160 mg/L, and retinol binding protein < 30 mg/L. The sTfR concentration ranged from 1.89 to 19.1 mg/L (mean: 8.7 mg/L). Mean values for indicators of nutritional status, serum ferritin, and transferrin saturation were within the normal range for healthy subjects. Regardless of the iron status (iron sufficiency, anemia, or iron deficiency with or without anemia) and whether women were pregnant or nonpregnant, undernutrition did not significantly reduce sTfR concentrations. A higher percentage (80%) of iron-deficient women with two or three protein values below normal had sTfR concentrations > 8 mg/L (which are suggestive of iron-deficiency erythropoiesis) compared with iron-deficient women with no (72.7%) or one (66.7%) protein value below normal, anemic women (46–60%), and iron-sufficient women (18.2–36.8%). Results suggest that sTfR can be used as an indicator of iron deficiency in field studies without in-depth assessment of nutritional status. However, the effect of severe malnutrition on this index requires further investigation. *Am J Clin Nutr* 1996;63:596–601.

**KEY WORDS**  Transferrin receptor, iron deficiency, ferritin, hemoglobin, undernutrition, albumin, prealbumin, retinol binding protein, Zaire

**INTRODUCTION**

Iron deficiency remains a public health problem in children and in women of childbearing age throughout the world. The problem is more common in developing countries, where as many as 60% of the children in some populations may be affected (1, 2). Its diagnosis usually involves the measurement of several variables, but the most common are hemoglobin and serum ferritin. In pure iron-deficiency anemia, both serum ferritin and hemoglobin concentrations are below specific cut-off points for sex and age (3). Serum ferritin concentrations < 12 µg/L are always suggestive of depletion of body iron stores. However, in the anemia of chronic disease, serum ferritin concentration may be increased even if body iron stores are inadequate (4, 5). In fact, when inflammation, infection, liver diseases, or other chronic diseases are present, iron deficiency cannot be ruled out even when serum ferritin concentrations are as high as 50 or 100 µg/L (3–5). Other indexes, such as free erythrocyte protoporphyrin, hemoglobin, and transferrin saturation, are not spared by these conditions. These problems are very common in central Africa, where iron deficiency is also recognized to be high (6–8). The use of measurements not significantly (or not at all) affected by these conditions is therefore required for the evaluation of the prevalence of iron deficiency in population studies.

Transferrin receptor, a disulfide-linked transmembrane glycoprotein, plays an essential role in cellular iron uptake, especially in bone marrow (9). Although it is a cell membrane protein, small quantities circulate in blood and are called soluble transferrin receptor (sTfR). Recent work by different investigators has suggested that sTfR is a sensitive indicator of tissue iron deficiency (10–13). Its blood (plasma) concentrations are increased several fold in subjects with iron deficiency, whereas they remain within the normal range in those with iron overload (10–14). Plasma sTfR concentrations are not affected by pregnancy unless the subject is also iron-deficient (15). Work by Ferguson et al (13) suggested that, unlike serum ferritin, sTfR is not affected by infection and inflammation and it may distinguish the anemia of chronic disease from that due to iron deficiency (13). However, in developing countries where this indicator might be useful, mild or moderate malnutrition is very common (16–19). The effects of undernutrition on sTfR concentrations have not been previously reported. This study was therefore designed to determine the influence of undernutrition on sTfR concentrations in iron-sufficient and iron-deficient women of reproductive age.

**SUBJECTS AND METHODS**

**Subjects**

The study was approved by the Ethics Committee of the Nsundi-Lutete Hospital (Bas-Zaïre State). Informed consent

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was obtained before blood collection. The initial study population included 160 Zairean women ranging in age from 16 to 45 y (x ± SD: 26 ± 6.7 y). After assessment of inflammation by measuring acute-phase proteins [α1 acid glycoprotein and C-reactive protein (CRP)], 61 women were eliminated. The data reported in this paper were therefore based on 99 women: 46 lactating women, 32 pregnant women, and 21 nonpregnant, nonlactating women. Because lactation per se does not affect iron status, data for lactating women and nonpregnant, nonlactating women were combined. The blood samples used in this study were collected during the dry season (summers of 1986 and 1989) in rural Bas-Zaïre (Sub-Region Luozzi) ~450 km southwest of Kinshasa during a survey on the prevalence of anemia and iron deficiency in women of childbearing age. The plasma samples were kept at -40 °C. Full information on criteria of recruitment and results on the prevalence of anemia and/or iron deficiency were published previously (20).

Laboratory methods

Plasma sTfR was measured by enzyme immunoassay using a commercial kit manufactured and sold by R&D Systems (Minneapolis). Briefly, the following was done: test samples and controls were diluted 1:100 in phosphate-buffered saline and standards were added to duplicate wells of a 96-well plate precoated with anti-human transferrin receptor. The plate was incubated at 37 °C for 2 h. After decanting the fluid, anti-sTfR-horseradish peroxidase conjugate was added to all wells and the plate was further incubated at 37 °C for 2 h. The second incubation was followed by washing the plate with phosphate-buffered saline. An enzyme substrate (tetramethylbenzidine) was then added to each well, and the plate was incubated at room temperature for 30 min. The reaction was stopped by adding sulfuric acid, and the absorbance was determined in a Bio Rad Microplate (enzyme-linked immunosorbent assay) Reader, (model 3550; Richmond, CA) set at 450 nm. The concentrations of test samples were determined from a standard curve prepared with each assay. To test the reliability of sTfR kits, three control plasma samples kindly provided by the laboratory of JD Cook (University of Kansas, Kansas City) were assayed in each experiment. In addition, nine samples were assayed two to three times 4–12 mo after the first measurement. Although the kits detected lower than expected concentrations of the control samples, the mean CV was < 6%.

Nutritional status was assessed by the measurement of transport proteins that are either specific (albumin) or sensitive [prealbumin (also called transthyretin) and retinol binding protein (RBP)] indicators of protein-energy undernutrition. Prealbumin and RBP are considered sensitive indicators because they respond very rapidly to protein and energy restrictions as well as supplementation; however, they are also decreased by inflammation or infection (21–24). Albumin is less sensitive but is more specific than both prealbumin and RBP because a decrease in its blood concentration is usually associated with changes in other indicators of malnutrition, such as body weight and skinfold thickness. These proteins were measured by radial immunodiffusion using antibodies, standards, and control plasma purchased from Behring Diagnostics (Somerville, NJ). Although transferrin was also assayed, it was not used in the classification of malnutrition because it is affected by both iron deficiency and undernutrition.

In addition to nutritional status, iron status was also assessed by measuring the following indicators: hemoglobin (by the cyanmethemoglobin method), serum ferritin (by radioimmunoassay), serum iron, and total-iron-binding capacity (TIBC) (by colorimetric methods). In nonpregnant women, hematocrit was also measured. To rule out inflammation, which is known to decrease the concentration of hemoglobin and biochemical indicators of nutritional status or to increase serum ferritin concentrations, two acute-phase proteins were measured by radial immunodiffusion: α1 acid glycoprotein (AGP) and CRP. Inflammation was defined as either an AGP concentration > 1.2 g/L or a CRP concentration > 50 mg/L (25).

Undernutrition was diagnosed if the concentrations of any of the three transport proteins were below the following cutoff points previously defined in the literature (26): albumin < 35 g/L, prealbumin < 160 mg/L, and RBP < 30 mg/L. Three categories of undernutrition were defined: 1) well-nourished (concentrations of all three proteins were within the normal range), 2) one protein below normal range, and 3) two or three proteins below the normal range. For each category of undernutrition, women were further subdivided according to their iron status: anemic (with normal serum ferritin concentrations), iron-deficient with or without anemia, and normal or iron-sufficient. Anemia was diagnosed if hemoglobin concentrations were < 110 g/L for pregnant women and < 120 g/L for nonpregnant women. Iron deficiency was diagnosed if serum ferritin concentrations were < 12 μg/L and/or ferritin saturation (ferritin saturation = serum iron/TIBC × 100) was < 16% together with a serum iron concentration < 9 μmol/L (500 μg/L) (3, 27). sTfR concentrations > 8 mg/L were considered abnormal and suggestive of iron-deficiency erythropoiesis. This cutoff point was based on our previous observation in 13 iron-sufficient laboratory employees who showed an upper limit of 7.26 mg/L (28), as well as the observations of other investigators (13).

Statistical analysis

Descriptive statistics (mean and SD), analysis of variance (ANOVA), and chi-square test were calculated with the use of a microstatistical program (Eco-Soft Inc, Indianapolis, IN) and as described in the literature (29). This program is similar to the SAS Statistical Package. For serum ferritin and sTfR, data were first transformed by decimal logarithm (log10) to achieve normality before performing the ANOVA. When the ANOVA detected significant differences in the mean concentration of any measurement, Scheffé’s test was also performed. The level of significance was set at α = 0.05.

RESULTS

Results for indicators of iron and nutritional status are summarized in Table 1. The mean sTfR concentrations of the overall study population were slightly above those reported in the literature for iron-sufficient subjects (13) as well as those we observed in iron-sufficient female laboratory employees in New Orleans (28). In parallel, the mean hemoglobin concentrations were below normal according to the criteria of the World Health Organization (27). The mean as well as the range of sTfR concentrations were similar between pregnant and nonpregnant women. Mean concentrations of hemoglobin, se-
TABLE 1
Iron and nutritional status of the study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>All women (n = 99)</th>
<th>Nonpregnant women (n = 67)</th>
<th>Pregnant women (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sTfR (mg/L)</td>
<td>8.70 ± 3.50 (1.88–19.1)</td>
<td>8.84 ± 3.47 (2.60–19.66)</td>
<td>8.41 ± 3.62 (1.89–17.6)</td>
</tr>
<tr>
<td>Log sTfR</td>
<td>0.905 ± 0.181</td>
<td>0.915 ± 0.170</td>
<td>0.882 ± 0.209</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>115 ± 16.6</td>
<td>114.1 ± 15.9</td>
<td>117 ± 17.8</td>
</tr>
<tr>
<td>Hematocrit (1)</td>
<td>0.381 ± 0.031</td>
<td>0.381 ± 0.031</td>
<td>ND</td>
</tr>
<tr>
<td>Serum ferritin (µg/L)</td>
<td>47.1 ± 39.9</td>
<td>57.4 ± 40.8</td>
<td>25.4 ± 25.9</td>
</tr>
<tr>
<td>Log ferritin</td>
<td>1.517 ± 0.394</td>
<td>1.663 ± 0.285</td>
<td>1.211 ± 0.419</td>
</tr>
<tr>
<td>Serum iron (µmol/L)</td>
<td>18.02 ± 10 [95]</td>
<td>18.3 ± 10.8 [63]</td>
<td>17.5 ± 8.58</td>
</tr>
<tr>
<td>TIBC (µmol/L)</td>
<td>85.3 ± 15.7 [98]</td>
<td>83.9 ± 17.8 [63]</td>
<td>88.2 ± 9.80</td>
</tr>
<tr>
<td>Transferrin saturation (%)</td>
<td>21.8 ± 13.5 [95]</td>
<td>22.8 ± 15.0</td>
<td>19.85 ± 9.74</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>41.6 ± 9.51</td>
<td>46.2 ± 7.89</td>
<td>31.9 ± 3.62</td>
</tr>
<tr>
<td>Transferrin (mg/L)</td>
<td>213.2 ± 81.3</td>
<td>233.0 ± 78.5</td>
<td>171.7 ± 73.3</td>
</tr>
<tr>
<td>Retinol binding protein (mg/L)</td>
<td>33.1 ± 13.0</td>
<td>34.0 ± 11.46</td>
<td>31.15 ± 13.11</td>
</tr>
<tr>
<td>Transferrin (g/L)</td>
<td>3.89 ± 1.05</td>
<td>3.44 ± 0.73</td>
<td>4.86 ± 0.98</td>
</tr>
</tbody>
</table>

1 SD; n in brackets. sTfR, soluble transferrin receptor; TIBC, total-iron-binding capacity.
2 Range.
3 Significantly different from nonpregnant women, P < 0.001 (ANOVA).

Regardless of the iron status (adequate iron status, anemia, or iron deficiency with or without anemia) in the overall study population, undernutrition did not significantly affect the mean concentration of sTfR (Table 2). When data from women with different iron statuses were combined, we also found no significant effect of undernutrition on the concentration of sTfR. Although the difference was small and not statistically significant, iron-sufficient women tended to have lower mean sTfR concentrations than either anemic or iron-deficient women. This trend was generally true regardless of the number of proteins that were below the normal range. When nutritional status was not taken into account, we observed that iron-sufficient women had significantly lower concentrations of sTfR than iron-deficient women (P < 0.05, Scheffé’s test). In pregnant and nonpregnant women, despite the small sample size in some of the cells, undernutrition did not significantly affect the mean concentrations of sTfR (Table 3). This was also true whether or not iron status was taken into account.

According to our previous observation in laboratory employees at Louisiana State University in New Orleans (28) and those of other investigators (13), the upper limit of sTfR for iron-sufficient subjects (men and women) was ~7.3 mg/L.

TABLE 2
Concentrations of soluble transferrin receptor (sTfR) in the overall study population as a function of iron status and undernutrition

<table>
<thead>
<tr>
<th>Number of protein concentrations below normal</th>
<th>Iron-sufficient</th>
<th>Anemic</th>
<th>Iron-deficient with or without anemia</th>
<th>All women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(4.87–11.42)²</td>
<td>(5.36–17.79)</td>
<td>(5.49–18.36)</td>
<td>(4.87–18.36)</td>
</tr>
<tr>
<td></td>
<td>(1.89–17.1)</td>
<td>(2.60–16.96)</td>
<td>(3.94–19.1)</td>
<td>(1.89–19.1)</td>
</tr>
<tr>
<td>All women'</td>
<td>7.48 ± 2.74 [36]</td>
<td>8.40 ± 3.60 [22]</td>
<td>9.92 ± 3.71 [41]'</td>
<td>8.70 ± 3.50[99]</td>
</tr>
</tbody>
</table>

1 SD; n in brackets. No significant effect of undernutrition was observed between mean sTfR concentrations.
2 Range.
3 Values in first three columns are significantly different from one another, P < 0.01 (ANOVA).
4 Significantly different from iron-sufficient women, P < 0.05 (Scheffé’s test).
TABLE 3
Concentrations of soluble transferrin receptors as a function of iron status and undernutrition in pregnant and nonpregnant women

<table>
<thead>
<tr>
<th>Iron status and number of protein concentrations below normal</th>
<th>Nonpregnant women</th>
<th>Pregnant women</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron-sufficient</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>8.06 ± 2.00 [19]</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>7.65 ± 4.08 [8]</td>
<td>3.73 ± 3.0 [3]</td>
</tr>
<tr>
<td>2 or 3</td>
<td>—</td>
<td>7.30 ± 1.12 [6]</td>
</tr>
<tr>
<td>Iron-deficient and/or anemic women ²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10.78 ± 4.34 [16]</td>
<td>7.98 ± 0 [1]</td>
</tr>
<tr>
<td>2 or 3</td>
<td>10.72 ± 0.45 [4]</td>
<td>9.23 ± 3.55 [15]</td>
</tr>
</tbody>
</table>

² x ± SD; n in brackets.

Because of the small sample size in some of the cells, data for women with any indicators of iron status below normal were combined. There were no significant differences among women with similar iron status but with different numbers of plasma protein concentrations below the normal range.

Assuming that 7.3 mg/L is the actual lowest concentration of sTfR for iron-deficient women in the studied population, the percentage of misclassified anemic and iron-deficient women was determined (Figure 1). In the iron-deficient group, ≈20% of undernourished women compared with 0% of well-nourished women were misclassified. Approximately the same proportion of women with one protein concentration or two to three protein concentrations below normal were misclassified. However, the difference between these proportions was not statistically significant by the chi-square test. In the anemic group, however, ≈36% of undernourished women were misclassified, with no significant difference between women with one protein concentration or two to three protein concentrations below normal. It is possible that the anemia of those women misclassified was due to causes other than iron deficiency. The chi-square test could not be used because no anemic woman had all three protein concentrations in the normal range.

The percentage of women with sTfR concentrations > 8 mg/L, suggestive of iron-deficiency erythropoiesis, was determined in the overall study population as well as in subgroups of women with different iron statuses and/or nutritional status (Figure 2). In the overall population, regardless of their nutritional status, a slightly lower percentage of iron-sufficient women than either anemic women or iron-deficient women had sTfR concentrations > 8 mg/L (Figure 2A). Although the sample size was small in some of the groups, the same trend was observed in pregnant (Figure 2B) and nonpregnant (Figure 2C) women. There were no significant differences (by chi-square test) between the proportions of women with sTfR concentrations > 8 mg/L with and without iron deficiency and undernutrition.

DISCUSSION

In many central African countries undernutrition is a common problem in children (16–19) and adults, especially in
women (30, 31). In adults, undernutrition is manifested by a low body mass index (in kg/m$^2$; < 18) as well as low serum concentrations of indicators of nutritional status (30, 31). The present study suggests that 31, 23, and 42 of the 99 women had albumin, prealbumin, and RBP concentrations below normal, respectively. Although no subject had severe malnutrition (albumin concentrations < 21 g/L), 25 women (25.3%) had at least two transport proteins in the range suggestive of undernutrition. Because no women with inflammation were included, the low concentrations of these transport proteins could only be attributed to undernutrition.

Considering that sTIR is a plasma protein, we wished to determine whether undernutrition would affect (decrease) its blood concentration and therefore confound its interpretation as an indicator of iron status. Although undernutrition (defined as one or more plasma protein concentrations below normal) was slightly related to lower mean concentrations of sTIR, in no case was the difference significant. In addition, when the analysis included only women with iron deficiency, the mean sTIR concentrations of women with two or three protein concentrations below normal were identical to those of women with three protein concentrations in the normal range. Even after pregnancy and iron status were taken into account, the trend was maintained—undernutrition did not significantly affect sTIR concentrations (see data for nonpregnant women, Table 3). One possible explanation for the lack of a significant relation is the fact that the main source of sTIR is the bone marrow, not the liver, which is the major site of synthesis for biochemical indicators of malnutrition. It is also thought that unlike transport proteins (indicators of malnutrition), circulating transferrin receptors are not secreted but rather sloughed off the cell membranes of the erythron (9). It is possible that in mild or moderate malnutrition, the bone marrow is not affected significantly enough to impair erythropoiesis and hence sTIR concentrations. However, in severe malnutrition, the bone marrow may be affected to such a degree that erythropoiesis is reduced and sTIR concentrations are decreased. This would explain the tendency of decreased sTIR concentrations in anemic as well as iron-deficient women with undernutrition (Table 2). In iron-deficient women, however, undernutrition did not reduce sTIR concentrations even in those women with three protein concentrations below normal compared with those with all three protein concentrations in the normal range, which suggests that the effect of iron deficiency on the sTIR concentration is more important than that of undernutrition. This, in turn, suggests that in fieldwork the majority of iron-deficient subjects with undernutrition may still be correctly identified by this index. Our results may imply that once cutoff points for normal concentrations have been established, this index may be used in field studies without in-depth assessment of nutritional status. However, our observation needs to be corroborated in a larger sample size and in subjects of different age ranges with different degrees of malnutrition, eg, kwashiorkor and/or marasmus.

We thank James D Cook for providing us with control plasma samples, which allowed us to determine the reliability of the sTIR assay.

REFERENCES


