

Serum Transferrin Receptor in Relation to Iron Status in Different Disease Stages of Adult Malignant Lymphoma

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ABSTRACT

Background: Soluble transferrin receptor is a new diagnostic tool for determining iron status and erythropoietic activity. The objective of this study was to evaluate the diagnostic efficiency of serum soluble transferrin receptor (sTfR) measurements together with the estimation of bone marrow iron stores in different phases of non-Hodgkin's lymphoma (NHL), in an attempt to assess the iron status of NHL patients.

Patients & Methods: The study was carried on 75 adult patients diagnosed as NHL at the National Cancer Institute, Cairo University. Hematological studies and bone marrow (BM) aspirate examination for cellularity, sideroblasts and marrow iron stores were done. Iron metabolism investigations including serum iron, iron-binding capacity, % saturation, ferritin and transferrin receptors (sTfR) were carried out. All the parameters were evaluated at different NHL phases of disease and compared in anemic and non-anemic patients.

Results: A significant increase in sTfR and BM iron stores was found in new and relapsing cases and in advanced stages of the disease, with normal levels in patients in remission. The difference between anemic and non-anemic NHL patients was mainly in levels of serum ferritin and TfRs and in BM iron stores.

Conclusions: This study showed elevated sTfR levels in adult NHL patients in spite of having abundant iron stores, and that its level reflected the activity and stage of the disease rather than iron status in this type of hematological malignancy.

Key Words: Soluble transferrin receptor (sTfR) - Non-Hodgkin's lymphoma (NHL) - Serum iron - Ferritin - Transferrin.

INTRODUCTION

The transferrin receptor circulating in plasma is a truncated form of the dimeric cellular transferrin receptor that is shed from cells [19]. In normal subjects, about 80% of the cellular TfR are found on erythroid precursors in the bone marrow and roughly the same proportion

of the circulating sTfR is derived from these erythroid cells. Accordingly, erythroid marrow activity is the principle determinant of the serum TfR concentration [4]. If erythroid hyperplasia can be excluded, an increase in the sTfR concentration is highly specific for tissue iron deficiency [14]. Unlike the serum ferritin concentration, the sTfR concentration is not increased in infection, inflammation or liver disease. Thus, the serum sTfR concentration provides a means of detecting iron deficiency even in the presence of chronic inflammation or infection and for distinguishing the anemia of iron deficiency from the anemia of chronic disease [4].

It was previously concluded that host iron status could influence the incidence and intensity of neoplasia, as it was found that iron withholding represented a defense mechanism against infection and neoplasia [10]. The potential utility of iron deprivation treatments as components of cancer therapy was confirmed by the selective inhibitory effects on lymphocyte activation, which were produced by monoclonal antibodies against the TfR [13]. Therefore, in the present study, we measured the circulating sTfR in malignant lymphoma patients at different phases of the disease, in addition to bone marrow iron stores and serum iron studies to assess the iron status of NHL patients in relation to the stage of the disease.

PATIENTS AND METHODS

This study was carried out on 75 patients diagnosed as NHL at different phases of the disease. They were adult patients presenting to the out-patient clinic of the medical oncology de-

partment of the National Cancer Institute, Cairo University during a two-year period (1996-1997). Their ages ranged from 16 to 68 years. The male to female ratio was 1.5: 1. Twenty of the 75 cases were newly diagnosed cases (group 1), 30 patients were studied during receiving chemotherapy (group 2), 15 patients were in complete remission (group 3) and 10 patients were in relapse (group 4). Ten healthy adult individuals of comparable age, sex and socioeconomic status were considered as controls.

Clinical examination for B symptoms, signs and staging of the disease was evaluated. Peripheral blood samples were obtained from patients and controls and divided into 2 parts: one part was added to EDTA and the other was for serum separation. The EDTA blood was used to perform the following hematological investigations:

Complete blood picture, including hemoglobin (Hb), red blood cell count, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Reticulocyte count (RC) & reticulocyte index (RI) [7].

Serum was separated and stored at - 20°C for the study of:

- Liver and kidney functions.
- Serum iron and total iron binding capacity (TIBC) [12].
- Ferritin utilizing a quantitative immunoenzymatic assay by kits supplied by Sorin Biomedica, Italy.
- Transferrin receptors were evaluated by a quantitative sandwich enzyme immunoassay technique using kits purchased from Quantikines, TM, USA [9]. The sample was taken in the early morning to overcome diurnal variation.

Anemia was defined as (Hb) of <12.8 g/dl for men and 11.7 g/dl for women [16]. Bone marrow aspiration was studied for marrow cellularity and normoblast percent within marrow differential count, iron stores and sideroblasts count by iron stain: (Perl's reaction) [7]. The evaluation of iron stores was rated on the following scale, 0 or 1 = no (rating 0) or only small (rating 1) granules detectable in reticulum cells at 1,000x magnification; 2 or 3 = small granules

detectable in reticulum cells at 400x (rating 2) or 100x (rating 3) magnification; 4,5, or 6 = iron visible with large granules in small clumps (rating 4), dense, large clumps (rating 5), or very large deposits (rating 6).

Statistical Methods:

The statistical analysis system (SAS) was used for data management and analysis. Harvard graphics package was used for the figures. Data was summarized as means and standard deviations. For comparisons between the different factors and stage of the disease, Mann-Whitney test, a non-parametric test equivalent to the Student t-test, was used. Correlation between quantitative variables was done using Spearman Rank correlation. Comparison was done between anemic and non-anemic NHL patients by the Student-t-test. P-values <0.05 were considered significant. All reported p-values were two-sided [1].

RESULTS

None of the patients had blood loss or endocrine disorders. Disseminated lymphoma was diagnosed in ten of the patients; 5 of group 1, 3 in group 2, 1 in each of groups 3 and 4. Liver functions were elevated in 7 (9.3%) of the patients especially those on chemotherapy. Elevated creatinine concentration of > 2 mg/dl was detected in 2 (2.6%) patients. The mean and standard deviation of hematological features observed in the different studied groups are shown in table-1. Hemoglobin level below 13 g/dl for males and 12 g/dl for females was observed in 10,22,0 and 8 cases of groups 1,2,3, and 4 respectively. Hemoglobin below 10.5 g/dl was found in 4,11 and 6 of groups 1,2 and 4; while Hb below 8 g/dl was found in 1,7 and 3 of the same groups, respectively.

Results of iron studies, and sTfR concentration observed in different patient groups are summarized in table (2). Fig. (1) is illustrating the serum TfR levels in the different studied groups. On comparing the different parameters studied within each group, we found that sTfR and serum iron are significantly related to the serum ferritin levels within the healthy controls ($p = 0.02$ and 0.04 , respectively). Serum ferritin was significantly related to serum iron within group 2 ($p = 0.005$), while sTfR concentration was significantly related to serum iron in group 3 ($p = 0.0001$).

All parameters studied were analysed in relation to the stage of disease and results are listed in table (3). Table (4) represents the results of different parameters in anemic and non-anemic lymphoma patients. On comparison of anemic versus non-anemic patients within the total studied NHL cases, it was found that the difference between both groups lies in marrow iron stores, ferritin and sTfR levels ($p = 0.001, 0.002$ and 0.042 , respectively), being elevated in anemic patients.

Based on bone marrow examination using iron staining as the acknowledged standard for iron deficiency, 14/40 anemic NHL cases showed low bone marrow iron stores rating (+1, +2), while the remaining 26 anemic patients had high iron stores rating (+3, +4, +5). None of our 40 patients showed absence of stainable iron in bone marrow examination. Serum TfRs was elevated in both types of patients those with high or low iron stores, with a mean score of 4.7.

Table (1): Hematological data of patients and controls.

Data	Control	Group I	Group II	Group III	Group IV	p-value
Hb (g/dl)	13.4±1.3a	11.1±1.8bc	10.2±2.3c	12.5±2.2ab	9.5±2.6c	<0.001
RBC (x 10 ¹² /L)	4.6±0.5	4.3±0.6	4.1±0.7	4.3±0.6	3.9±0.8	0.094
Hct (%)	40.5±2.5a	34.2±5.1c	32.7±6.4bc	38.6±5.1ab	31.0±7.9c	<0.001
MCV (fl)	88.1±6.3a	78.0±10.2c	77.4±9.2c	87.5±16.5ab	77.1±11.7bc	<0.001
MCH (pg)	29.1±3.5	25.8±3.5	24.6±4.3	29.4±4.7	25.0±3.5	NS
MCHC (g/dl)	33.5±3.8	32.3±3.1	31.2±4.0	32.8±2.9	31.0±3.2	NS
RC (%)	0.4±0.3a	2.0±1.6b	1.1±1.3b	1.2±1.1b	2.8±3.1b	0.004
RI (%)	0.4±0.2a	1.5±1.2b	0.7±0.9b	0.8±0.8b	1.8±1.8b	0.004

- All values are mean ±SD.
- p- values less than 0.05 are considered significant.
- For each significant test group means sharing the same letter are not significantly different than each other.

Table (2): Iron studies and TFRs levels in the studied groups.

Data	Control N=10	Group I N=20	Group II N=30	Group III N=15	Group IV N=10	p-value
Serum Fe (µg/dl)	95.7±44.8a	77.4±80.4	93.7±52.4	75.2±40.2	75.8±38.0	0.087
TIBC (µg/dl)	295.4±120.7	222.9±141.2	322.5±136.9	251.6±93.1	281.3±8.4	0.060
Transf. sat (%)	35.4±14.2	41.5±28.8	33.5±20.0	35.3±18.6	29.0±22.4	0.568
TFRs (µg/ml)	3.8±0.6	4.6±1.6	4.6±1.3	3.6±0.9	4.2±1.4	0.059
Ferritin (µg/l)	46.6±25.3b	490.3±676.6a	634.4±692.9a	267±538.8ab	441.6±597±0a	0.001
Sideroblasts (%)	—	3.7±3.0ab	1.4±1.3c	14.2±12.8a	2.1±3.1bc	0.001
Iron stores (score)	—	3.1±0.9b	2.8±1.3bc	1.9±0.8c	2.6±1.0bc	0.001

- All values are mean ±SD.
- p- values less than 0.05 are considered significant.
- For each significant test group means sharing the same letter are not significantly different than each other.

Table (3): All parameters studied in relation to stage of the disease.

Data	Stage I-II n=35	Stage III-IV n=36	p-value
Age, years	21.9±19.8	30.8±19.6	0.027
Hb (g/dl)	10.7±2.0	10±2.2	0.451
RBC (x 10 ¹² /L)	4.2±0.6	4.1±0.7	0.201
Hct (%)	33.3±5.6	33.1±6.6	0.849
MCV (fl)	76.3±9.2	78.8±9.8	0.165
Rc (%)	1.2±1.3	1.6±1.6	0.299
RI (%)	0.9±0.9	1.1±1.2	0.524
Fe (µg/dl)	81.1±60.0	95.2±66.8	0.424
TIBC (µg/dl)	299.6±142.6	276.2±149.8	0.538
Transf. sat (%)	34.6±32.7	38.0±326	0.465
TFRs (µg/ml)	4.2±1.2	4.9±1.5	0.025
Ferritin (µg/l)	379.6±560.5	798.6±745.6	0.175
Fe store (score)	2.5±1.1	3.4±1.1	0.002

* p- value < 0.05 is considered significant.

Table (4): Comparison of parameters studied in anemic and non-anemic NHL patients.

Data	Anemic N = 40	Non anemic N=35	p-value
Age, years	22.3±18.0	30.3±18.9	0.736
Hb (g/dl)	8.9±1.6	12.9±1.2	0.088
RBC (x 10 ¹² /L)	3.7±0.6	4.5±0.3	0.002
Hct (%)	29.2±4.9	39.6±3.2	0.006
MCV (fl)	75.2±10.7	84.1±8.4	0.113
MCH (pg)	1.7±2.0	1.4±1.3	0.005
MCHC (g/dl)	26.3±5.6	26.1±3.9	0.210
Rc (%)	31.3±3.4	31.7±3.0	0.381
RI (%)	1.1±1.2	1.0±1.1	0.950
Serum iron (µg/dl)	80.9±571	95.6±56.9	0.990
TIBC (µg/dl)	276.9±123.7	290.6±128.6	0.787
Transf. sat (%)	34.4±21.7	37.3±23.3	0.547
TFRs (µg/ml)	4.6±1.5	3.8±1.0	0.042
Ferritin (µg/l)	758.2±692.1	220.1±425.9	0.002
Fe stores (score)	3.0±1.0	2.0±1.0	0.001
Creatinine (mg/dl)	0.8±0.3	0.7±0.2	0.564

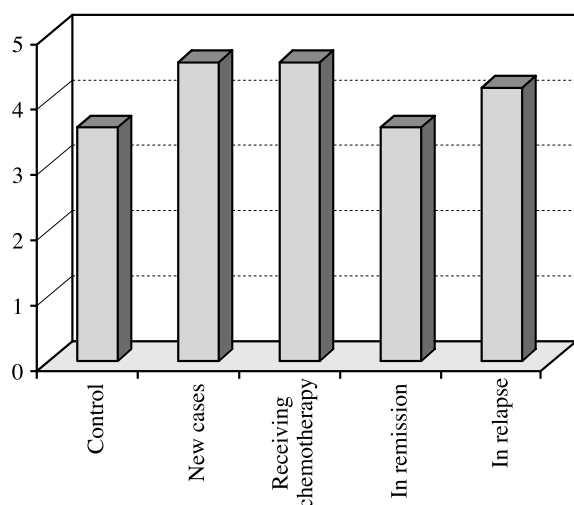


Fig. (1): TFR levels in NHL patients.

DISCUSSION

The importance of accurate assessment of iron status in chronically ill patients is clearly evident in 2 different situations. It is necessary to identify patients with true iron - deficiency anemia, even in the presence of complicating factors, since the treatment of these patients with iron supplementation is effective. However,

to optimize the use of supplemental iron in treatment of anemic conditions other than iron - deficiency anemia, it is necessary to also identify patients with functional iron deficiency (FID) i.e. iron-deficient erythropoiesis in the presence of adequate or increased iron stores. The clinical significance of FID becomes most important in that it is a major factor limiting the efficacy of rHuEPO treatment [1,6].

The conventional tests to analyze iron status (serum level of iron, total iron-binding capacity, and transferrin and ferritin levels) are not useful for detecting IDA in complicated anemic patients [18]. Serum ferritin reliably predicts iron deficiency anemia in an uncomplicated anemic setting [5]. However, the prediction is of limited value in lymphoma patients because of increased ferritin levels as part of the acute phase response [17]. A significant correlation of serum ferritin with CRP and LDH in lymphoma patients was previously reported [17]. Herein, we describe measurement of sTfR as an additional tool to detect iron status in adult anemic NHL patients.

Measurement of sTfR concentration is a helpful new laboratory method for the diagnosis of iron deficiency [14]. TfRs mediate cellular iron uptake, and their expression is up regulated when the cellular demand for iron is increased. In uncomplicated IDA, the serum concentration of TfR has been shown to differentiate between iron-depleted and iron-repleted anemic states, irrespective of the presence of acute or chronic inflammatory conditions [7].

Serum levels of sTfR were found to be elevated in our lymphoma patients as new cases, during receiving their initial chemotherapy and at relapse when compared to controls and to NHL patients at a remission ($p= 0.059$). Moreover, elevation of sTfR concentration was more pronounced in advanced stages of disease, as higher levels of sTfR were detected in stages III and IV when compared with stages I and II. Thus, we consider that sTfR in adult lymphoma patients go hand in hand with disease activity. Similarly Medeiros et al. [15] stated that late stage lymphomas express more sTfR than early stage lymphoma and it can be considered as a marker for proliferation. A recent study showed that elevated sTfR levels in patients with malignancy correlated with severity of the disease but not with iron stores [2].

On comparing sTfR levels of our patients in relation to their Hb concentration, a statistically significant higher sTfRs were detected in anemic cases when compared with non-anemic patients ($p=0.04$). Also there was a significant difference between the two groups regarding iron stores ($p = 0.001$) denoting high iron status.

As regards the bone marrow iron stores of our anemic lymphoma patients, none of them showed absence of stainable iron in the bone marrow. Punnonen et al. [16] previously defined iron deficiency in their patients as complete absence of stainable iron in BM. Accordingly, none of our patients had iron deficiency. Similarly, previous reports stated that patients with acute leukemia and lymphoma have high serum iron levels and high levels of transferrin-saturation [10]. It was also demonstrated by the Prussian blue reaction in their erythroblasts in the BM [3].

In spite of having adequate marrow iron stores, our NHL patients have increased TFRs. This is in agreement with other studies reporting that hematological malignancies are associated with an elevated serum TfR regardless of the iron status of the patients [11]. Furthermore, it was concluded that soluble TfRs could not be considered a good parameter for making a diagnosis of iron deficiency in chronic diseases [8].

It is important to notice that in our new NHL patients, in spite of having abundant iron stores (score 3.1 ± 0.9) when compared to patients in remission (score 91.9 ± 0.8), still an inverse relationship between iron stores and % sideroblast was observed. The % sideroblast was 3.7 ± 3.0 in new cases and 14.2 ± 12.8 in patients in a remission. BM iron stores and % sideroblast were significantly different in both groups with a p -value of 0.001 for both variables. It is evident that in patients with active disease, although having a high iron status, this iron was not incorporated into sideroblasts.

Thus, in view of these findings, we conclude that sTfRs are not suitable to diagnose the iron status in adult NHL patients and rather could either reflect an increased marrow iron stores or indicate an increased cellular demand for iron. Further research is needed for the combined measurement of sTfR together with tumor TfR to evaluate the source of increased serum soluble transferrin receptors.

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