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Serum Transferrin Receptor Assay in Iron-Deficiency Anemia and Anemia of Chronic Diseases

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Abstract. Serum Transferrin Receptor is a biochemical parameter used for the detection of iron deficiency in situation where ferritin has limited diagnostic value. This study investigates the level and the significance of serum transferrin receptor among patients with iron deficiency anemia and anemia of chronic diseases at King Abdulaziz University Hospital in Jeddah, Saudi Arabia. All samples were collected from patients, ages ranged between 20-63 years, from May to December 2008. Seventy three control subjects (61 males and 12 females), plus 65 anemic patients were enrolled into this study, which was divided into two groups: 37 (3 males and 34 females) patients with iron deficiency anemia and 28 (15 males and 13 females) patients with anemia of chronic diseases. Anemia of chronic diseases patients, were divided into sub-categories of malignant diseases (n=11), chronic inflammatory disease (infectious and non-infectious) (n=9) and an end stage renal disease (n=8). Complete blood counts, serum ferritin, serum iron, serum transferrin receptor and serum transferrin receptor/log ferritin were measured for these subjects. The mean results of the serum transferrin receptor concentration among control subject were 2.47±0.62 μg/mL (range 1-3.65 μg/mL). All patients with iron deficiency anemia had an elevated serum transferrin receptor level (>3.65 μg/mL); chronic diseases had normal levels of serum transferrin receptor; except 8. The higher value in these patients suggests the presence of concurrent iron deficiency. There

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were no significant differences in the mean of serum transferrin receptor concentration and sex in control subjects and two groups of patients. A significantly higher serum transferrin receptor/log ferritin was observed in iron deficiency anemia (9.33 ± 8.81) compared to control subjects (1.32 ± 0.39) and anemia of chronic diseases (1.35 ± 1.1). The mean of serum transferrin receptor concentration and serum transferrin receptor/log ferritin in anemia of chronic diseases were not significantly different from that of the control subjects. There was no significant differences between different causes of anemia of chronic diseases: malignant diseases, chronic inflammatory diseases (infectious and non-infectious) and end stage renal disease and mean serum transferrin receptor concentration (2.68±1.80 μg/mL, 2.38 ± 1.39 μg/mL, and 2.71 ± 2.11 μg/mL respectively). Therefore, using the serum transferrin receptor concentration is recommended to differentiate between simple iron deficiency anemia and anemia of chronic diseases, plus it is a very useful tool when anemia of chronic diseases coexists with iron deficiency anemia.

Keywords: Iron deficiency Anemia, Anemia of Chronic Disease, Serum transferrin Receptor.

Introduction

Iron Deficiency Anemia (IDA) is the most common micronutrient deficiency in the world. It may result from the lack of diet, chronic blood loss, increased requirement and mal- absorption. Globally, it affects 1.62 billion people, which corresponds to 24.8% of the population[1]. Anemia of chronic disease (ACD) is the most frequent anemia in hospitalized patients after IDA, which is the second most frequent anemia in the world. The prevalence and severity of anemia are correlated with the stage of the underlying condition, and it appears to increase with advanced age. Anemia of chronic diseases involve immune driven cytokines cells of the reticuloendothelial system that secrete cytokines such as interferon-γ, TNF-α and IL-6, which induce changes in iron homeostasis. A major effect is the up-regulation of Hepcidin mainly by IL6 expression by the liver, which reduces iron absorption and contributes to its sequestration on the reticuloendothelial system. Other sequelae includes reduction of the proliferation of erythroid progenitor cells, the production of erythropoietin, and the reduction of the life span of red cells (decreased sensitivity to it), all of which contribute to the pathogenesis[2].

Serum transferrin receptor (sTfR) is a truncated form of the intact receptor, lacking its first 100 amino acids (cytoplasmic and transmembrane domains), which circulates in the form of a complex of

transferrin and its receptor. The main sources of sTfR are the erythropoietic cells of the bone marrow, including circulating reticulocytes. The amount of circulating sTfR is proportional to the total amount of cell-associated transferrin receptor. The concentrations of sTfR correlate directly with erythropoietic activity, and inversely with the amount of iron available for erythropoiesis, providing a quantitative measure of functional iron status [3].

Anemia in patients with inflammation may be difficult to evaluate as conventional laboratory measurements of iron status are often unable to differentiate between iron deficiency anemia and anemia of chronic disease due to ferritin. This could be increased as it's acute phase reactant making it necessary to do a bone marrow examination to evaluate iron store[4].

Serum transferrin receptor is a biochemical parameter used for the detection of iron deficiency in situation where ferritin has limited diagnostic value owing to the present chronic disease [5]. The serum transferrin receptor blood test may be a better indicator of iron status as it is not affected by inflammation [6].

Objectives

Accordingly, this study investigates the level and the significance of serum transferrin receptor among patients with iron deficiency anemia and anemia of chronic disease at King Abdulaziz University Hospital (KAUH) in Jeddah province.

Materials and Methods

The study was carried out on 73 control subjects (61 males and 12 females) after an informed consent agreement. Samples were randomly collected in EDTA from clotted blood of healthy blood donors at KAUH, Jeddah. All samples of subjects were collected from May to December 2008. Their ages ranged between 20-63 years old. Complete blood count (CBC), serum ferritin (SF), serum irons (SI) and serum transferrin receptor (sTfR) were performed at all samples. Two groups of anemic patients were included IDA and ACD in this study. In the IDA group, 37 EDTA and clotted blood samples (3 males, 34 females) were collected from hematology clinics. In addition, 28 EDTA and clotted blood samples (15 males and 13 females) from ACD patients were collected

from Medical Wards, which was subdivided into malignant disease (n = 11), chronic inflammatory disease (infectious and non-infectious) (n = 9) and an end stage renal disease (n = 8). Study patients were classified as having IDA (hemoglobin: < 12 g/dl for females, < 14 g/dl for males; serum ferritin: < 13 mg/L for females, < 30 mg/L for males), or ACD (hemoglobin < 12 g/μL for females, < 14 g/dL for males). A complete blood count was measured using Beckman Coulter LH750 machine in the hematology laboratory at KAUH-Jeddah to determine CBC.

The electrochemiluminescence immunoassay (ECLIA) is ROCHE Elecsys immunoassay analyzer (ROCHE Diagnostics GmbH-D-68298 Mannheim) was used to get the ferritin level, while quantitatively determination of serum iron was carried out by using ROCHE/Hitachi automated clinical chemistry analyzers. Throughout the study, the entire mechanism was checked and calibrated by using standard quality assurance at the beginning of the experiment. The serum transferrin receptor was determined by enzyme-linked immune sorbent assay. The measured concentration of samples and on quality controls calculated from the standard curve has to be multiplied by their respective dilution factor. Both, the sensitivity and specificity of sTfR in IDA were found to be 100%, whereas in ACD, these were 66.6% and 100%, respectively. The positive and negative predictive value, in case of IDA was 100%, whereas in ACD it was 100% and 74.1%, respectively.

Serum transferrin receptor ferritin index was calculated as the ratio to the logarithm of ferritin concentration (sTfR (mg/mL)/ log ferritin (mg/L)[7].

Statistical Analysis

In this study, the data were analyzed using the SPSS computer program version 15.0.1 package. All results were reported as means of ± SD, and the one way ANOVA test was used to assess different causes of ACD with sTfR concentration. Differences between two groups were compared using an independent *t*-test; statistical significance was indicated at $p \leq 0.05$.

Results

In the present study, 73 EDTA clotted samples were collected from healthy males and females blood donors at KAUH-Blood Bank,

considered as control subjects 61 (83.6%) males and 12 (16.4%) females. Their ages ranged between (20-63 years).

Complete blood count, sTfR, SF and SI were performed in the hospital to all the samples of control subjects. These samples showed normal hemoglobin levels, serum ferritin and iron.

The reference ranges of the control subjects of the investigated parameters of hematological and biochemical variables are shown in Table 1.

Table 1. Reference ranges of the investigated parameters of hematological and biochemical variables in the control subjects (n=73).

Investigated Parameters	Reference Ranges	
	Male	***Female***
Hb	14 -18 g/dl	12-15 g/dL
Serum ferritin (SF)	30 - 400 µg/L	13 - 150 µg/L
Serum iron (SI)	10.6 - 28.3 µmol/L	6.6 - 26.0 µmol/L

Hb = hemoglobin level

Sixty-five blood samples were collected from patients in the same hospital (hematology clinics), and were divided into two groups. The first group included 37 patients with IDA. Their ages ranged between 20 - 63 years. Thirty-four (91.9%) patients were females and 3 (8.1%) were males.

The second group of patients (n = 28) had ACD, blood samples were collected from medical wards of the same hospital. Their ages ranged between 20 - 63 years. Fifteen (53.6%) patients were males and 13 (46.4%) were females. Table 2 shows the comparisons between control subject, patient with IDA and ACD in the investigated parameters using mean and standard deviation.

Table 2. Comparisons between control subject, patient with the IDA and ACD in the investigated parameters using mean and standard deviation.

Investigated Parameters	Control Subject Mean ± SD	IDA Mean ± SD	ACD Mean ± SD
Hb	14.75 ± 1.18	10.20 ± 1.65	9.08 ± 1.94
MCV	85.01 ± 3.76	71.69 ± 8.43	83.63 ± 9.93
MCH	28.41 ± 1.56	23.05 ± 3.39	28.00 ± 4.15
Serum Iron	15.78 ± 4.76	7.81 ± 4.75	12.36 ± 10.64
Serum Ferritin	95.43 ± 75.45	6.69 ± 3.96	260.98 ± 249.46

Hb-hemoglobin level ; MCV- Mean corpuscular volume; MCH- mean corpuscular hemoglobin

The mean of sTfR receptor for control subjects was 2.47 ± 0.6 (range 1-3.65) as presented in Table 3.

Table 3. The mean of serum transferrin receptor (sTfR) concentration and sTfR/Log F, plus it is p value in control subjects and two groups of patients (ACD and IDA).

Groups	sTfR (µg/ml)		sTfR/log F	p value
	Range	Mean ± SD		
Control	1-3.65	2.47±0.62	1.32±0.39	
ACD	1.1-6.1	2.59±1.72	1.35±1.11	0.945
IDA	4.1-6.7	5.29±0.77	9.33±8.81	0.000

p≤0.05 was used as a criterion of significance.

In IDA patients, the mean of sTfR concentration was 5.29 ± 0.77 µg/mL (range 4.1-6.7 µg/mL), while the mean concentration in the ACD patients was 2.59 ± 1.72 µg/mL (range 1.1-6.1 µg/mL) as shown in Table 3.

In patients with ACD, the sTfR level was higher in 8 (28.6%) patients compared with that of control group (Fig. 1).

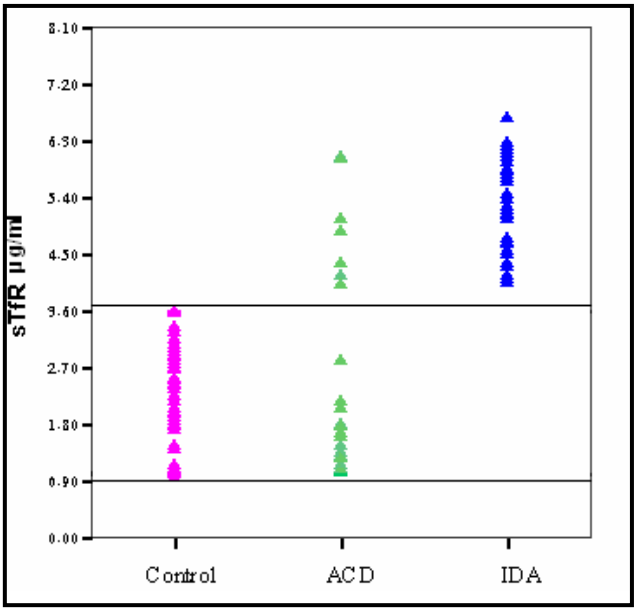

Fig. 1. Serum transferrin receptor levels in control subjects; Anemia of Chronic Diseases (ACD) and Iron Deficiency Anemia (IDA).

Patients with IDA showed a significantly higher sTfR/ log F compared with the controls, (p = 0.000). Whereas, patients with ACD had no significant difference in sTfR/Log F compared with that of the controls (p = 0.945) as illustrated in Table 3.

Table 4 represents the relationship between serum transferrin receptor and sex in the control subjects, and two groups of patients in (IDA and ACD). The mean of sTfR concentration in Control males was 2.48 ± 0.59 μg/mL compared with 2.09 ± 0.81 μg/mL in control females.

Table 4. The relationship between serum transferrin receptor and sex in control subjects and two group of patients (IDA and ACD).

Groups	Sex	sTfR (μg/ml)	P - value
Control	Male	2.48 ± 0.59	
	Female	2.09 ± 0.81	0.06
IDA	Male	4.75 ± 0.35	
	Female	5.33 ± 0.78	0.214
ACD	Male	2.77 ± 1.86	0.57
	Female	2.39 ± 1.6	

p≤0.05 was used as a criterion of significance.

In IDA patients, the mean of sTfR concentration for males and females were 4.75 ± 0.35 μg/mL and 5.33 ± 0.78 μg/mL, respectively. Also, the mean of sTfR concentration was 2.77 ± 1.86 μg/mL and 2.39 ± 1.6 μg/mL in male and female patients with ACD, respectively. There were no significant differences between mean of sTfR concentration and sex in control subjects and two groups of patients (IDA and ACD) as shown in Table 4.

Table 5 represents the relationship between sTfR and the different causes of ACD. Eleven patients had malignant diseases (39.3%), 9 patients had chronic inflammatory diseases (infections and non-infectious (32.1%) and 8 patients had end stage renal disease (28.6%) represented in the ACD group. The mean concentration of sTfR in malignant diseases, chronic inflammatory diseases (infections and non-infectious) and end stage renal disease were 2.68 ± 1.80 μg/mL, 2.38 ± 1.39 μg/mL and 2.71 ± 2.11 μg/mL, respectively. There were no significant differences between mean sTfR concentration and the different causes of ACD as shown in Table 5.

Table 5. The relationship between serum transferrin receptor and different causes of anemia of chronic diseases.

Investigated Parameter	Type of Chronic Diseases	n	%	Mean ± SD	p-value
sTfR µg/ml	Malignant diseases	11	39.3	2.68±1.80	0.909
	Chronic inflammatory diseases (infectious and non-infectious)	9	32.1	2.38±1.39	
	End stage renal disease	8	28.6	2.70±2.11	

$p \leq 0.05$ was used as a criterion of significance.

Discussion

Anemia remains a widespread public health problem with major consequences for human health as well as social and economic development[1]. The most severe consequence of iron depletion is IDA, and it is still considered as the most common nutrition deficiency worldwide.

The ACD is the most frequent anemia in hospitalized patients and develops in subjects suffering from chronic inflammatory disorders (infectious and non-infectious), cancer and end-stage renal disease[2].

The evaluation of anemia in patients with inflammation may be difficult as conventional laboratory measurements of iron status are often unable to differentiate between IDA and ACD. Because ferritin could be increased as it's an acute phase reactant, therefore, it is necessary to do a bone marrow examination to evaluate iron stores and to establish a definitive diagnosis. Nevertheless, this examination cannot be routinely performed since it is invasive, painful, expensive, plus time consuming[8].

During the recent years, the measurement of sTfR concentration has been introduced as a new diagnostic tool to detect iron deficiency, as well as to differentiate between anemia caused by iron deficiency and that caused by chronic diseases [6].

Transferrin receptor (TCR) is a 188-KDa polypeptide chain while sTfR is truncated 85-KDa form of the whole sTfR molecule [9]. The increased levels of sTfR are found in patients with IDA, and normal or low levels are found in patients with ACD. Patients with the ACD may also have concomitant true iron deficiency and then, may show sTfR levels similarly elevated as in pure iron deficiency anemia.

In the present study, the mean of sTfR concentration among control subjects was 2.47 μg/mL (range 1-3.65 μg/mL), which agreed with manufacturer's stated normal range of 0.9 to 3.3 μg/mL. Our results showed that the mean values of sTfR were higher in patients with IDA than those in control, and in patients with ACD (5.29 ± 0.77; 2.47 ± 0.62; 2.59 ± 1.72 μg/mL, respectively). This is consistent with other studies by Genc *et al.*[10] and Keskin *et al.*[11]. In both studies, sTfR was found higher in patients with IDA than those in patients with ACD and the control subjects.

Moreover, all patients with IDA had elevated sTfR levels (> 3.65 μg/mL) and patients with ACD had normal levels of sTfR, except 8 patients which had increased sTfR levels. As sTfR is usually not elevated in ACD, the higher value in these patients suggests the presence of concurrent iron deficiency[6].

In all groups, the gender did not significantly affect the concentration of sTfR. There is no clear explanation for this finding in the literature. In our opinion, this could be explained by the fact that number of receptors do not change with sex. This is compatible with other studies by Margetic *et al.*[12], Vernet and Doyen[13] where they found no significant differences between male and female in the values of sTfR in control subjects. Furthermore, Punnonen *et al.*[14] also showed no significant differences in sTfR concentration between male and female patients of (IDA and ACD).

The ratio between sTfR and ferritin concentration (sTfR/log F) was proposed as a good indicator for evaluating iron deficiency. This is as it presents relationship between an increase in sTfR and a decrease in ferritin concentration in iron deficiency. Two variables are influenced by the body iron stores, the availability of iron for erythropoiesis, and the total mass of the erythroid progenitors in the bone marrow.

A ratio of less than 1 suggests anemia of chronic disease, whereas a ratio of more than 2 suggests absolute iron deficiency coexisting with ACD or pure IDA. A ratio between 1 and 2 is indeterminate, and further evaluation may be required, including an iron stain of the bone marrow, to better assess the possibility of coexisting iron deficiency [7].

The present study revealed highly significant differences between in sTfR/log F between IDA and control subjects. Similar observation have

been reported by Markovic *et al.*[15], when compared several hematological and biochemical variables, between IDA and control subjects. Moreover, the ACD showed no significant difference in sTfR/log F compared to control, while the IDA and ACD, showed a significant difference ($p < 0.05$) in sTfR/log F.

The present study, disclosed no significant differences between the mean sTfR concentration in malignant diseases (2.68 ± 1.80 μg/mL), chronic inflammatory diseases (infections and non-infectious, 2.38 ± 1.39 μg/mL) and end stage renal disease (2.71 ± 2.11 μg/mL). The different categories of diseases among ACD will lead to the same kind of anemia, with similar pathogenesis, this might explain the lack of significant difference in the p-value. No similar studies were found in the literature.

In conclusion, the sTfR concentration is a useful serum marker of iron depletion, increased erythropoiesis as well as rapid cell proliferation. It is a useful investigation to diagnose ACD when associated with IDA when ferritin level is high, and there is a diagnostic dilemma. This parameter should be used in conjunction with the traditional parameters that measures iron depletion.

Recommendations

Unlike what is known in the literature in regards to the high cost of sTfR assay, the Eliza kit is relatively cheap and comparable to ferritin kit. Therefore, recommendation in using this diagnostic assay, especially when the differentiation between simple IDA and ACD is problematic, is therefore, advisable. In addition, it is a very useful diagnostic tool when ACD coexist with IDA.

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قياس مستقبل الدم الترانسفيرين في فقر الدم الناجم عن نقص الحديد وفقر الدم الناجم عن الأمراض المزمنة

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المستخلص. مستقبل مصل الدم الترانسفيرين هو معيار كيميائي حيوي يستخدم للكشف عن نقص الحديد في الحالة التي يكون فيها الفرتين محدود القيمة التشخيصية بسبب مرض مزمن. مستقبل مصل الدم الترانسفيرين قد يعتبر مؤشرا أفضل لوضع الحديد لأنه لا يتأثر بالالتهابات. وفقا لذلك فقد صممت هذه الدراسة لقياس مستقبل مصل الدم الترانسفيرين وأهميته في فقر الدم الناجم عن نقص الحديد وفقر الدم الناجم عن الأمراض المزمنة في مستشفى جامعة الملك عبد العزيز في محافظة جدة. في هذه الدراسة، تم جمع جميع عينات الأشخاص من مستشفي جامعة الملك عبد العزيز من جماد الأولى إلى ذو الحجة من عام ١٤٢٩هـ، وتراوحت أعمارهم بين (٢٠-٦٣ عاما). ثلاث وسبعين من الأشخاص الضابطين (٦١ ذكور و١٢ إناث) و٦٥ مريضا بفقر الدم تم تسجيلهم في هذه الدراسة، وتنقسم إلى مجموعتين: ٣٧ (٣ ذكور و٣٤ إناث)، المرضى الذين يعانون من فقر الدم الناجم عن نقص الحديد و ٢٨ (١٥ ذكور ١٣ إناث) المرضى الذين يعانون من فقر الدم الناجم عن الأمراض المزمنة. أيضا في المرضى الذين يعانون من فقر الدم الناجم عن الأمراض

المزمنة، تم تقسيم المرضى، إلى فئات فرعية من الأمراض الخبيثة (عددهم ١١)، والأمراض الالتهابية المزمنة (المعدية وغير المعدية) (عددهم ٩) والمرض الكلوي في المرحلة النهائية (عددهم ٨). وفي هذه الدراسة تم قياس عدد خلايا الدم الكامل، حديد مصل الدم، فرتين مصل الدم، مستقبل مصل الدم الترانسفيرين و النسبة بين مستقبل مصل الدم الترانسفيرين ولوغارتيم الفرتين. أظهرت النتائج أن متوسط تركيز مستقبل مصل الدم الترانسفيرين بين المجموعة الضابطة كان 2.47 ± 0.62 ميكروجرام / مل (المدى ١ – 3.65 ميكرجرام/مل). جميع المرضى الذين يعانون من فقر الدم الناجم عن نقص الحديد لديهم مستويات مرتفعة في مستقبل مصل الدم الترانسيفيرين والمرضى الذين يعانون من فقر الدم الناجم عن الأمراض المزمنة لديهم مستويات طبيعيه باستثناء ٨ مرضى قد زادت عندهم مستويات مستقبل مصل الدم الترانسفيرين. يقترح أن يكون ارتفاع القيمة في هؤلاء المرضى بسبب وجود نقص الحديد المتزامن. لا توجد فروق بشكل ملحوظ بين متوسط تركيز مستقبل مصل الدم الترانسفيرين ونوعيه الجنس في المجموعة الضابطة ومجوعتي المرضى (فقر الدم الناجم عن نقص الحديد وفقر الدم الناجم عن الأمراض المزمنة). في فقر الدم الناتج عن الأمراض المزمنة متوسط تركيز مصل الدم الترانسفيرين والنسبة بين مستقبل مصل الدم الترانسفيران ولوغارتيم الفرتين ليست مختلفة بشكل ملحوظ عن المجموعة الضابطة. ولا يوجد هناك اختلافات بشكل ملحوظ بين الأسباب المختلفة لفقر الدم الناجم عن الأمراض المزمنة (الأمراض الخبيثة)، والأمراض الالتهابية المزمنة (المعدية وغير المعدية) والمرض الكلوي في المرحلة النهائية وبين متوسط تركيز مستقبل مصل الدم الترانسفيرين (2.68 ± 1.80 ميكروجرام / مل، 2.38 ± 1.39 ميكروجرام / مل و 2.71 ± 2.11 ميكروجرام / مل على التوالي).

لذا نوصي باستخدام تركيز مستقبل مصل الدم الترانسفيرين خصوصا عندما يكون التفريق بين فقر الدم البسيط الناجم عن نقص الحديد وفقر الدم الناجم عن الأمراض المزمنة صعباً. بالإضافة إلى ذلك، يعد أداة تشخيصية مفيدة جدا عندما يكون فقر الدم الناجم عن الأمراض المزمنة ملازماً لفقر الدم الناجم عن نقص الحديد.