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Evaluation of diagnostic efficacy of serum sTfR assay in iron-deficiency anemia and Beta-thalassemia trait in Shafa Hospital, Ahvaz, Iran 2010

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Abstract. – BACKGROUND AND OBJECTIVES: Soluble form of transferrin receptor (TFR) called soluble TFR (sTfR) is shed mainly from the erythroid precursors and with a slower rate from other tissues into the plasma. This process of release is intensified in situations characterized with a some degree of erythroid hyperplasia or body iron stores depletion, such as seen in beta-thalassemia trait (betaTT) and iron-deficiency anemia (IDA), respectively. Therefore, the employement of sTfR assay as a diagnostic tool for differentiating IDA from betaTT in case of co-existence of these two clinical entities seems to be questionable. In this work we decided to study the above-mentioned dilemma in our geographical area, south of Iran.

MATERIALS AND METHODS: Whole blood (5 ml) and serum samples (2 ml) were collected from 30 patients with IDA, 30 individuals with betaTT and 30 apparently healthy cases as control group. Complete blood count (CBC) was done by blood analyzer and serum iron, serum ferritin and serum sTfR were assayed by biochemical, immunological (chemiluminescence) and Elisa Kit, respectively.

RESULTS: Serum ferritin concentration in IDA group was significantly lower than the concentration seen in betaTT: 6.93 ± 4.16 vs 47.40 ± 32.33 µg/ml. The findings for sTfR serum concentration in IDA group (3.25 ± 1.60 microg/ml) and betaTT group (1.86 ± 0.36 microg/ml) showed a significant difference between IDA and the control group ($p < 0.001$), with some overlap between IDA and betaTT groups. Serum ferritin concentration and serum sTfR concentration in the control group were (65.60 ± 58.53 microg/dl) and (1.51 ± 0.22 microg/ml), respectively. The sTfR/ferritin ratio clearly showed a diagnostic superiority to ferritin assay in IDA diagnosis.

CONCLUSIONS: The observed overlap in serum stfR concentrations between IDA and betaTT groups makes the sTfR assay unefficient tool for a differential diagnosis between IDA and betaTT in the early stages of IDA. An higher diagnostic potential was observed in the advanced stage of iron deficiency anemia. Calculated ratio of serum sTfR/ferritin showed the diagnostic superiority to ferritin assay alone in IDA diagnosis.

Key Words:
Iron-deficiency anemia, Beta-thalassemia trait, Transferrin receptor.

Introduction

Iron is a vital natural element which participates in a vast variety of physiological processes in most living cells. Any shortage of dietary iron will affect iron body stores and if persists will cause a progressive declining change in body stores classified as deficiency, depletion and finally iron-deficiency anemia (IDA)[1]. During the earlier stage of this declining process in iron body store the laboratory signs remain unchanged or equivocal with the exception of serum soluble transferrin receptor (sTfR) concentration until the appearance of anemia in which most of the laboratory parameters become abnormal[2,3]. The diagnosis of isolated IDA, is an easy task but if paralleled with inflammation and β-thalassemia trait (βTT), it becomes difficult endeavor in some instance[4-6].

In βTT a degree of ineffective erythropoiesis is present causing a moderate erythroid hyperplasia with an iron excess which is balanced by the increased iron excretion preventing the iron accumulation[7]. The characteristic change in laboratory indices seen in βTT resembles in most cases, those seen in IDA, i.e. red blood cells (RBC) morphology, RBC indices, etc. Therefore, the diagnosis of the IDA in the presence of βTT turns to be uneasy problem to be solved, using the traditional tests only[8].

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There are reports recommending the sTfR assay as an efficient tool in this respect[9]. Transferrin receptor (TfR) is a vastly distributed protein through body tissues with a highest density in erythroid cells. Its soluble form (sTfR) is a truncated membrane receptor which is shed in the plasma mostly from the maturating erythroid cells. The serum concentration of this soluble antigen increases in iron-deficiency state such IDA and also in any condition characterized with erythroid hyperplasia such βTT[10].

Since reports concerning the efficacy of sTfR assay in differential diagnosis of IDA from βTT are conflicting, we decided to investigate this dilemma in these geographical areas in which these two clinical entities were prevalent([11]).

Materials and Methods

Patients with Hb <12 g/dl, mean corpuscular volume (MCV) <80 fl, mean corpuscular hemoglobin concentration (MCHC) <27 pg, ferritin <12 µg/L, serum iron <50 µg/dl and transferrin saturation <15% were selected as cases with iron-deficiency anemia. Patients with Hb <12 g/dl, MCV >80 fl, MCHC <27 pg, serum ferritin >15 µg/l, transferrin saturation of >15% and Hb A2 >3.8% along with a relevant family history for βTT were chosen as β-TT group. The conditions such as inflammatory diseases, renal diseases, liver disorders, megaloblastic anemia, pregnancy, recent-blood transfusion, iron-supplementation, colono-rectal hemorrhagic diseases were considered as exclusion criteria. Thirty patients as IDA group and thirty cases as βTT group were selected as characterized above from patients attending Shafa Hospital, the main Hematology Centre of Ahvaz University, Iran. From June to October 2010 thirty, apparently healthy individuals with normal hematological indices (age and sex matched) were recruitted as control group.

The ethical approval was obtained from the Ethics Committee of the Jundishapor University of Medical Sciences.

Five milliliter blood samples were drown, two milliliter of which was add to a tube containing EDTA-K anticoagulant and the rest was clotted and the serum portion separated and frozen at-70^{c}.

Complete Blood Count (CBC) was done by Mindray-BC-5500 (P.R. China) analyzer. Hb fractionation was done by electrophoresis at pH 8.6 on cellulose acetate and the obtained Hb-A_2 result was confirmed by mini column Ion-exchange chromatography on Gold analyzer, England (Drew Scientific Limited, Cumbria, UK).

Serum iron and total iron-binding capacity (TIBC) measured by Randox kit on Alcyon autoanalyzer, Abbott, USA (Abbott Laboratories, Abbott Park, IL, USA). Ferritin serum concentration was estimated by chemiluminescence assay, Liasion, Rome, Italy. An ELISA kit (BioVendor, Research and Diagnostic Products, BioVendor-Laboratorni Medicina a.s., 61600 Brno, Czech Republic) and TECAN-Spectra reader (TECAN Austria GmbH, Grodig, Austria, E-mail: tecan-a@tecan.co.at), were employed for measurement of sTfR serum concentration.

Statistical Analyses

Statistical analyses were done using SPSS-18 software (SPSS Inc. Chicago, IL, USA). Independent-sample *t*-test and One-way ANOVA used for comparative study and the correlation study was done by correlate-Bivariat test. $p < 0.05$ was cosidered statistically significant.

Results

A group of 30 healthy individuals (13 males and 17 females) with mean age of 48 years was investigated as control group. Also 30 patients (14 males and 14 females) with mean age of 50 years and 30 cases (12 males and 18 females) with mean age of 48 years were studied as IDA and βTT groups respectively.

The obtained results for hematological parameters and Iron- status indices are presented in Tables I and II. the obtained range for sTfR serum concentration ($\chi\pm2SD$) in control group was 1.51±0.22 µg/ml. which correlate well with the manufacturer‘s stated reference interval of 1.0 to 2.9 µg/ml. In IDA group the obtained range for serum sTfR level was 3.25 ± 1.6 µg/ml ($\chi\pm2SD$) which was significantly higher than those seen in βTT and control groups ($p < 0.001$).

The obtained range (1.86±0.35 µg/ml) for sTfR serum level in βTT group showed to be lower than that seen in IDA group ($p < 0.001$); however, no significant difference with the control group was observed ($p > 0.05$). The findings in Table I and II showed that Hb, MCV and MCHC parameters were significantly lower in IDA and βTT groups as compared with the control group. The reduction in Hb concentration was more remarkable in IDA group than the value seen

Table I. Hematological parameters in the different study populations

Parameters	IDA	Thalassemia trait	Control
RBC	4.60 ± 0.46	5.37 ± 0.57	4.86 ± 0.48
Hb (g/dl)	9.78 ± 1.01*	10.65 ± 0.97	13.80 ± 1.43
Hct (%)	32.34 ± 3.18	34.14 ± 3.31	41.78 ± 3.61
MCV (fl)	69.33 ± 5.13*	63.98 ± 7.06*	85.74 ± 3.69
MCH (pg)	20.82 ± 2.35*	20.08 ± 2.40*	28.05 ± 1.26
MCHC (g/dl)	29.94 ± 1.31	31.46 ± 1.01	32.73 ± 1.15
RDW	16.92 ± 2.64*	15.16 ± 1.62*	12.67 ± 0.64
HbA2 (%)	2.12 ± .041	3.81 ± 0.91*	2.17 ± 0.30

in the βTT group ($p = 0.03$), whereas MCH values showed no significant difference in these two groups. Transferrin saturation, serum iron and ferritin levels were significantly lower in IDA group in comparison with βTT and control groups; the total iron-binding capacity (TIBC) value was significantly higher in IDA group ($p < 0.001$). A significant ($p < 0.01$) negative correlation was observed between sTfR and other parameters such as serum Hb and ferritin levels (Figure 1), whereas there was not detected between sTfR level and serum iron or TIBC levels ($p > 0.05$). Mean serum ferritin concentration obtained in IDA group was lower than values observed in βTT and control groups ($p = 0.0001$). This difference was not detected comparing the ferritin means obtained in βTT group with the control ($p > 0.05$). Serum iron concentration was significantly lower in IDA group in comparison with βTT and control groups ($p = 0.0001$).

Discussion

Bone marrow aspiration and the iron staining is the ultimate assay for diagnosis of IDA. Since this technique is expensive and very unpleasant for patient, other non-invasive laboratory assays such as ferritin, CBC etc. are routinely employed for this purpose. The co-existence of IDA with clinical entities characterized with laboratory hematological parameters overlapping with those seen in IDA, ie. βTT, creates a situation in which the differential diagnosis of IDA from the accompanying clinical conditions will be uneasy task to achieve[11,12].

The diagnostic efficiency of sTfR assay has been studied by several investigators and conflicting results have been reported[10,11].

Both conditions of IDA and βTT are highly prevalent in our region and their co-existence is not uncommon situation. Therefore, the main task of this project was focused on the evaluation of the efficacy of sTfR assay in differential diagnosis between IDA and βTT.

The soluble form of TfR called sTfR is shed in the plasma mostly from erythroid precursors and with lower intensity from other body tissues. So, its serum concentration is rised in any condition characterized with some degree of erythroid hyperplasia or depletion of body iron stores such as IDA and βTT, respectively[10].

In this work the rise seen in sTfR concentration in βTT group did not differ significantly from the

Table II. Iron status parameters in the different study populations.

Parameters	IDA	Thalassemia trait	Control
Iron (µg/dl)	30.93 ± 8.38*	5.37 ± 0.57	84.96 ± 23
TIBC (µg/dl)	428.33 ± 42.72*	373.10 ± 34.38	385.73± 1944
TS (%)	7.45 ± 2.86*	23.74 ± 5.77	220.8 ± 5.90
Ferritin (µg/L)	6.93 ± 4.16*	47.40 ± 32.33	65.60 ± 58.53
sTfR (µg/ml)	3.25 ± 1.60*	1.86 ± 0.35	1.51 ± 0.22
sTfR/ferritin ratio (µg/µg)	805 ± 781.45	62.42 ± 42.52	44.02 ± 33.76

*Significant difference.

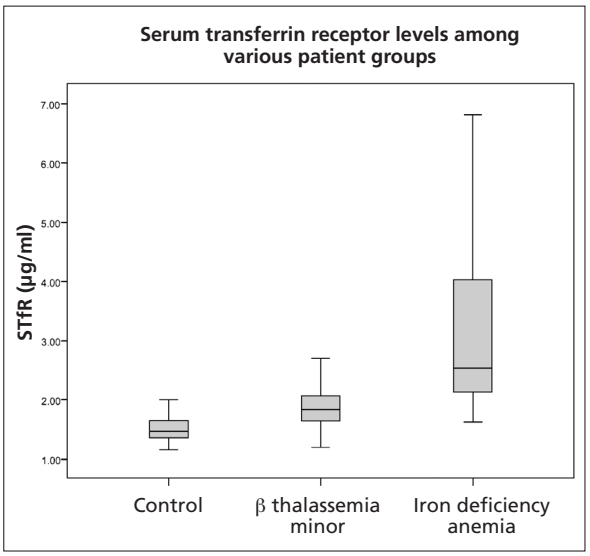

Figure 1. Boxplot graphs of sTfR levels in different groups of patients.

control group, although showed a relative mild elevation (Figure 2). This finding could be justified with the presence of a mild degree of erythroid hyperplasia usually seen in βTT. Although in the IDA group the sTfR serum concentration was significantly higher than in the control group (p = 0.0001), but showed some degree of overlap with a remarkable portion of the βTT group (57%). Our data showed a significant negative correlation between sTfR and Hb and ferritin serum concentration in IDA group (p = 0.016). These findings also showed that the overlapping phenomenon occurred between βTT group and patients from IDA group with a mild or moderate degree of iron-deficiency and not with the advanced stage of IDA. Therefore, we postulated that sTfR assay could be employed as a useful tool for a differential diagnosis between IDA and βTT only in advanced stage of IDA. This finding is in accordance with Ong et al[11] and disagrees with Skikne et al[13] and Suominen et al[14].

In contrast with Huebers et al[15] sTfR serum concentration showed no correlation with age in our caseload. As far as concerns the HbA2 relative concentration, our finding (Figure 3) agrees with the literature in this regard[8,16]: HbA2 relative concentration was significantly higher than the control group ($p < 0.0001$). Serum sTfR concentration of control group was in the recommended range (1-2.9 µg/ml). Only 42.9% of patients included in our IDA group were diagnosed as having truly IDA and the other 57.1% remains within the reference interval. In this respect, this portion of IDA group overlaps with βTT group (Figure 1). This finding agrees with Aysin et al[17]. Different reports were published by other investigators using other methods[18].

The diagnostic efficacy of serum ferritin concentration lower than the cut-off point of 12 g/l for IDA was referred in this investigation where we reported a diagnostic sensitivity and specificity of 83.3% and 96.7%, respectively. However, the diagnostic value of this assay is known to be

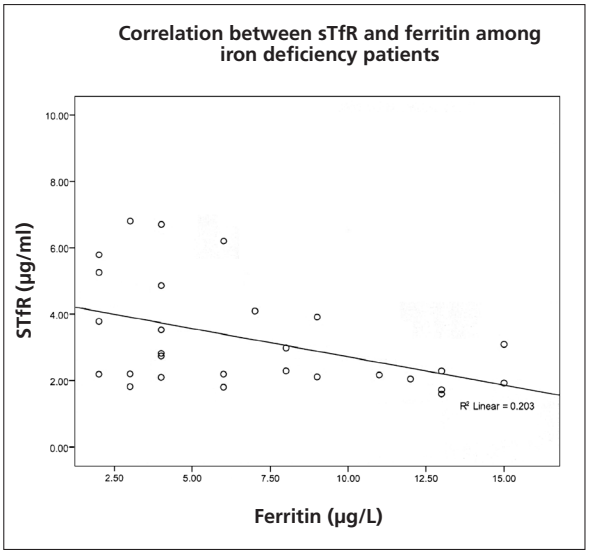

Figure 2. Serum transferrin receptorf (sTfR) levels versely correlate with serum ferritin ($p < 0.01$).

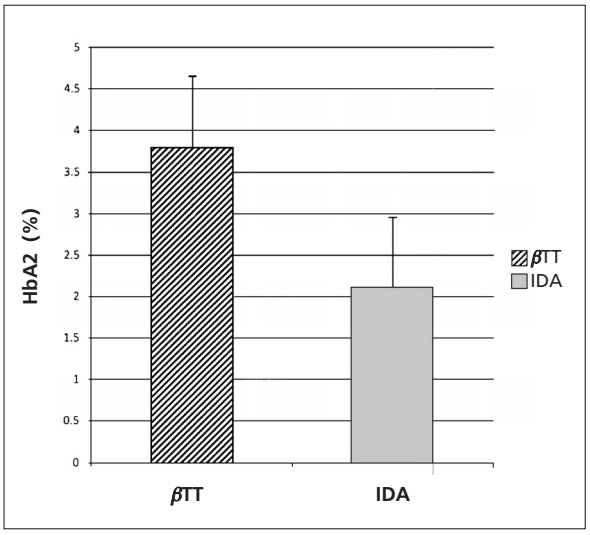

Figure 3. Relative percentage of HbA in β-thalassemia (BTT) and iron-deficiency anemia (IDA) groups.

susceptible to inflammatory processes, as has been demonstrated by Intragumtornchai et al[19].

The ratio of sTfR/Ferritin with a cut-off point of >130 μg/μg has been used as a tool for the diagnosis of IDA[6]. This index showed a diagnostic sensitivity and specificity of 92.9% and 93.1% respectively, clearly more efficient than serum ferritin assay.

In conclusion, our data showed that the sTfR assay allows a differential diagnosis between βTT and IDA only in the advanced stage of the latter.

Acknowledgements

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