

Methodology Evaluation of a New Immunoturbidimetric Method for Measuring Serum Soluble Transferrin Receptor

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Transferrin receptor (TfR)¹ is a glycoprotein, mediating the entry of ferric transferrin from the extracellular compartment into the cells. TfR is present on the surfaces of various cell types and it is most abundant on the cells functioning in hemoglobin synthesis. Up-regulation of the expression of cellular TfR occurs as a result of an inadequate tissue supply of the iron or an increased cellular demand for the iron. Therefore, an elevation of the soluble form of TfR (sTfR) in the serum can be detected in the context of iron deficiency anemia, thalassemia and polycythemia.¹⁻³

In clinical settings, sTfR measurements have been widely used and offer an attractive addition to the repertoire of indices of iron status. The sTfR concentration has also been shown to be a more sensitive and less variable index of the iron status than the more conventional parameters; i.e. serum iron, transferrin, and total iron-binding capacity.¹⁻³

Presently, the measurement

SUMMARY The value of soluble transferrin receptor (sTfR) detected in serum is closely related to erythroid TfR turnover rate. An increased erythropoietic activity causes an increase in the sTfR level. Therefore, it is a useful test for monitoring the erythropoiesis. In this study, a new immunoturbidimetric method for automated measurement of sTfR was evaluated for its performance characteristics. Imprecision studies on patients' sera with 1.01 mg/l and 2.94 mg/l concentrations yielded within-run CVs of 1.16% and 1.27%. Accuracy analysis of the test by using the low and high control kit sera with 1.45 mg/l and 5.41 mg/l concentrations were 89.06% and 95.41%, respectively. The evaluation was also performed in 60 individual pediatric subjects, 30 beta-thalassemia/HbE and 30 control pediatric subjects. There is a statistically significant difference of sTfR between both groups ($p < 0.0005$, 95% CI = 9.457-14.124). Ninety-five percent of matched pediatric subjects had sTfR level ≤ 2.670 mg/l and 93.33% of patients diagnosed beta-thalassemia/HbE had values > 2.670 mg/l. In conclusion, this immunoturbidimetric test yields good laboratory performance characteristics in terms of precision and accuracy.

of sTfR has become a widely used tool in assessing iron status, but its implementation has mainly been restricted to research laboratorial settings. A number of sTfR assays has been developed. Most of the recently developed sTfR assays are based principally on the radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).^{4,5} Recently, a particle enhanced immunoturbidimetric assay⁶⁻⁷ of soluble transferrin receptor (Orion Diagnostica, Espoo, Finland), which can be worked as a fully automated

sTfR assay has been introduced. This study was designed to evaluate the analytical performance characteristics of this new assay.

MATERIALS AND METHODS

Test principle

This assay is a particle enhanced immunoturbidimetric as-

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say⁶⁻⁷ of soluble transferrin receptor, IDeA sTfR-IT (Orion Diagnostica). The assay is based on the detection of an immunoreaction between sTfR and sTfR-specific antibodies in liquid phase. The immunoreaction is enhanced by particles coated with sTfR-antibodies. The measurement is performed by photometry at 660 nm. The amount of immunoprecipitate is proportional to the sTfR concentration in the sample. Several automated analyzers can be used to perform this assay. The measuring range of the test as recommended by the instruction's kit is 0.3 - 8.5 mg/l.^{6,7} The assay manual reported a correlation study between this immunoturbidimetric assay and an enzyme-linked immunosorbent assay (ELISA) with a correlation coefficient of 0.986 (n = 50).

Protocol for method evaluation

In order to evaluate some laboratory performance characteristics, protocol for linearity of measuring range, imprecision and inaccuracy tests was set. The linearity study was performed by serial two-fold dilutions of the serum sample with high sTfR level equal to 19.16 mg/l for four fold dilutions. The imprecision study was performed by determination of the within-run precision of 10 replicates. Within-run precision analysis of the test was performed using two patients' sera with low sTfR levels (sTfR levels = 1.01 and 2.94 mg/l). The inaccuracy study was performed by determination of % relative accuracy. The % relative accuracy analysis was performed using both low and high control kit sera (sTfR levels = 1.38 and 5.71 mg/l). The % relative accuracy was calculated by the formula: % relative accuracy = $\{1 - ([\text{mean value} - \text{actual value}] / \text{actual value})\} \times 100$. The acceptable %

relative accuracy must exceed 80%.⁸ Furthermore, inaccuracy was also indirectly studied as study of the reference value of sTfR in control subjects with normal hematological parameters and clinical correlation study in thalassemic patients as will be presented in the following context.

Subjects

Sixty pediatric subjects (age ranged from 2 years to 15 years) were included in this study and divided into 2 groups. The first group (N = 30) was the non-anemic group (normal red blood cell parameters determined by automated hematology analyzer, Technicon H*3) that served as a control group. This group was mainly composed of pediatric patients who were undergoing elective surgery at the King Chulalongkorn Memorial Hospital. The second group (N = 30) consisted of pediatric patients who were diagnosed thalassemia, the disease with the highest degree of erythropoiesis in human.⁹ All of the subjects in the second group were confirmed for beta-thalassemia/HbE by hemoglobin electrophoresis (Helena, Beaumont, Texas). The selection of the subjects was also made on the basis to exclude the concomitant effect of acute manifestation, iron deficiency, chronic inflammatory conditions, and dietary restrictions. Subjects of both groups were well matched by age and sex. Informed consent of all subjects was obtained from their parents before the study. All research protocols followed in this study are in accordance with the guideline of the local Ethical Committee (Faculty of Medicine, Chulalongkorn University).

Non-fasting blood specimens were collected from pediatric

subjects by antecubital venipuncture. Five milliliters of clotted blood was collected from each patient and sent to the Central Laboratory, King Chulalongkorn Memorial Hospital, Bangkok, Thailand. The collection of the samples was performed between 8.00 - 10.00 a.m. Sera were used for the laboratory analysis. All serum samples were stored at -20°C before thawing for further analysis within 6 months. Hemolytic, icteric and lipemic specimens were excluded.

Average sTfR levels (mean \pm 2 SD) for both groups of the subjects were calculated. Unpaired T-test was used for determination of statistical difference between average sTfR levels of the studied groups. The statistical significant level was accepted at a *p* value of 0.05. The expected range for serum sTfR levels for the healthy control group in this study was also determined as mean - 2 SD to mean + 2 SD. Statistical analyses were carried out using the SPSS 10 for Windows software (SPSS).

Laboratory analysis

All samples were analyzed for sTfR levels by the immunoturbidimetric assay kit using the automated clinical chemistry analyzer, Hitachi 911 (Roche-Boehringer Mannheim, Germany) for calibration technique. All analyses were performed according to the manufacturer's instruction.⁶⁻⁷ The method requires 20 μ l of sample, 250 μ l of IDeA sTfR-IT buffer, and 20 μ l of IDeA sTfR-IT reagent. The reagent consists of polyclonal anti-human TfR F(ab)₂ antibodies bound to SVBC-latex particles. In the presence of sTfR, the latex particles are agglutinated in a dose-dependent manner, causing increased turbidity.

ty. The amount of sTfR in the sample is determined by means of a calibration curve based on ready-to-use calibrators included in IDeA sTfR-IT test kit from Orion Diagnostica. Two additional controls (low and high controls, sTfR levels = 1.38 and 5.71 mg/l) are also supplied from Orion Diagnostica in the same test kit. Calibrators and controls were prepared from human serum source.

RESULTS

Evaluation of precision, accuracy and linearity

Within-run precision analysis of the test was performed using two patients' sera (sTfR levels = 1.01 and 2.94 mg/l). The results of the experiments are shown in Table 1. The within-run assay, % CV of 10 replicates was 1.158% and 1.270%. Accuracy of the test was performed using both low and high control kit

sera (sTfR levels = 1.38 and 5.71 mg/l). The percent accuracy of each level of control serum is presented in Table 2. The linearity of the method was tested by serially diluting serum samples (sTfR = 19.16 mg/l) to four fold dilutions, giving a broad range of linearity from 7.67 to 19.67 mg/l.

Evaluation of indirect accuracy by the mean of clinical correlation

The distribution of sTfR in the control group and the anemic thalassemic group are shown in Fig. 2. Average sTfR levels for the control group and the anemic thalassemic group were 1.894 ± 0.776 mg/l and 13.684 ± 6.209 mg/l, respectively. Expected reference range for sTfR level of the control from this study was 1.118 to 2.670 mg/l. There was a statistically significant difference of sTfR level between both groups ($p < 0.0005$,

95% CI = 9.457-14.124). From the clinical data correlation study, 95% of control subjects showed a sTfR ≤ 2.670 mg/l; 93.33% of patients with diagnosed beta-thalassemia/HbE had values > 2.670 mg/l.

DISCUSSION

Cellular iron uptake in human is mediated by transferrin receptors (TfR). A soluble form of TfR (sTfR) detected in the serum is closely related to the erythroid TfR turnover rate. Increased erythropoietic activity causes TfR synthesis to be upregulated and thereby increases sTfR level. Determinations of sTfR concentrations can reflect cellular iron demands and the erythroid proliferation rate.¹⁰ Therefore, measurement of sTfR has been introduced as a powerful tool for monitoring erythropoiesis in a variety of clinical situations.¹⁰⁻¹⁶

At present, several commercially available methods have been introduced to measure sTfR.^{16,17} Most of these available methods are enzyme immunoassays, which require a manual procedure. These methods are time-consuming and require medical scientist experienced in analysis to obtain valid results.⁵

Table 1 Within-run precision of the immunoturbidimetric assay for sTfR (N = number of replicates)

	N	Mean (mg/l)	SD (mg/l)	CV (%)
Patient serum 1 (1.01 mg/l)	10	1.014	0.012	1.158
Patient serum 2 (2.94 mg/l)	10	2.918	0.037	1.270

Table 2 Accuracy analysis of the immunoturbidimetric assay for sTfR (N = number of replicates)

	N	Mean (mg/l)	SD (mg/l)	CV (%)	Accuracy (%)
Low Control* (1.45 mg/l)	10	1.229	0.072	5.858	89.058
High Control* (5.71 mg/l)	10	5.448	0.160	2.937	95.412

*included in the test kit by Orion Diagnostica

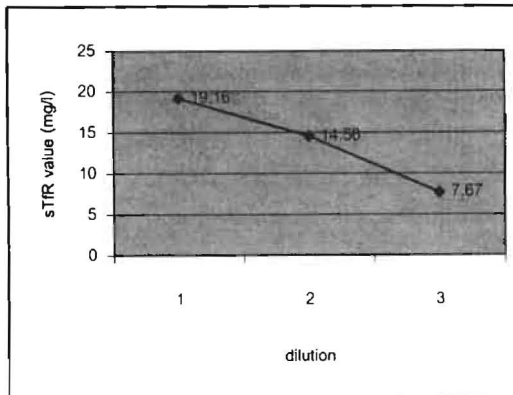


Fig. 1 The result from linearity study.

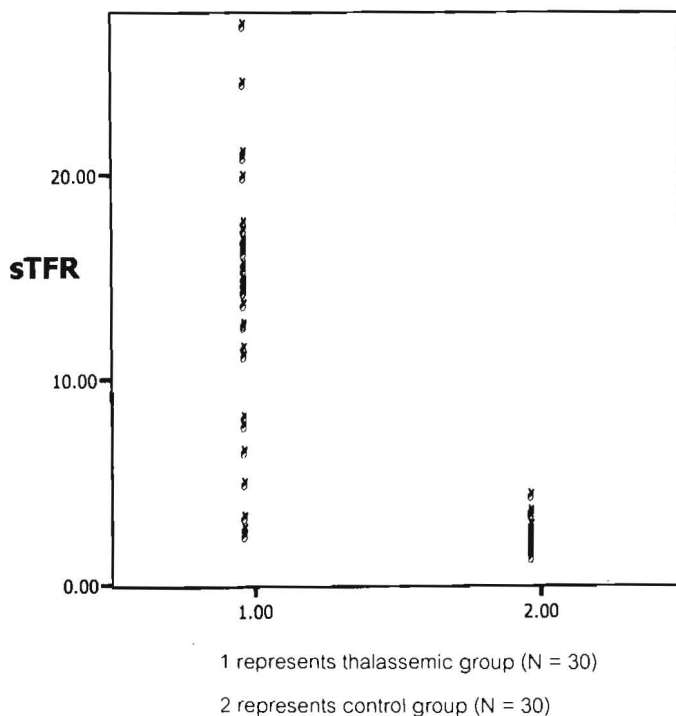


Fig. 2 The distribution of sTfR value (mg/l) in healthy control group and anemic thalassemic group.

This study was set to evaluate the analytical performance characteristics of a new automated immunoturbidimetric assay for sTfR, IdeA sTfR-IT (Orion Diagnostica) in our laboratory. This method can be used as a manual or automated immunoturbidimetric assay. In this study, we tested the IdeA sTfR-IT application on the Hitachi 911 analyzer (Roche-Boehringer Mannheim). The evalu-

ated test is also available for other automated clinical chemistry analyzers and can, therefore, be a convenient marker for monitoring erythropoiesis by a laboratory.^{18,19}

From analytical performance characters evaluation, this new test provided rather good precision (CV = 1.158 and 1.270% for the low sTfR level) and accuracy (89.058 and 95.412% for the low and high values). From a clinical data correlation study, 95% of control subjects sTfR were ≤ 2.670 mg/l; 93.33% of patients diagnosed beta-thalassemia/HbE had values > 2.670 mg/l.

The precision yield in our study is similar to those of previous reports evaluating the sTfR test kit of Suominen *et al.*⁷ and Wians *et al.*²⁰ Therefore, the precision of this test kit is acceptable and similar to other kits. Also, the same level of % relative accuracy was derived from this kit comparing to other kits.²⁰ Concerning the reference range (1.12 to 2.67 mg/l) derived from our study, a similarity to the previous reported reference range (0.41 to 1.94 mg/l) among the same age group²¹ was observed. The high sTfR levels in our thalassemic subjects also matched with the reported high values in the previous studies on thalassemic subjects.^{22,23}

Hence, the new immunoturbidimetric method described here can promote the usage of sTfR as another simple tool for monitoring erythropoiesis for clinical use and can be applied by any laboratory with automated analyzers.

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