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Evaluation of serum transferrin receptor for iron deficiency in women of child-bearing age

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The objective was to study the evaluation of serum transferrin receptor (sTfR) for Fe deficiency in women of child-bearing age. Primary screening was performed in 942 women ranging in child-bearing age. Serum ferritin (SF), Zn protoporphyrin (ZPP) and Hb were determined. Then the subjects were divided into four groups: normal, Fe store depletion (IDs), Fe-deficiency erythropoiesis and Fe-deficiency anaemia. sTfR was determined and sTfR/SF (sTfR/logSF and log(sTfR/SF)) was calculated. Changes of sTfR in women of different Fe status were observed. A receiver-operating characteristic (ROC) curve was used to evaluate whether sTfR had proper diagnostic efficacy for functional Fe deficiency. The levels of sTfR increased significantly along with the aggravation of Fe deficiency. Increase of STfR/SF along with the aggravation of Fe deficiency was more significant than that of sTfR. STfR had a significant negative correlation with SF and Hb, while it had a significant positive correlation with ZPP. The ROC curve showed that the diagnostic effective rate of sTfR for Fe deficiency could reach 83 %. At this point, the sensitivity was 79 % and the specificity was 63 %. Log(sTfR/SF) could be considered to have the highest effective ratio in detecting IDs, since it reached 99 %. STfR and sTfR/SF could both reflect body Fe-deficiency status specifically. They could be used as reliable indicators for evaluating Fe status and diagnosing Fe deficiency in women of child-bearing age.

Serum transferrin receptor: Iron deficiency: Women of child-bearing age: Diagnostic efficiency

Fe-deficiency anaemia (IDA) is a public health problem throughout the world, particularly in developing countries[1]. Fe deficiency is especially prevalent among infants, children, pregnant women, women of child-bearing age and senior citizens. The transition from normal levels to the development of IDA involves two sequential processes: Fe store depletion (IDs) and Fe-deficiency erythropoiesis (IDE)[2]. There are no additional physiological phenomena associated with the development of IDs and IDE, so they are classified as subclinical Fe deficiency. After exhaustion of the stored Fe compartment, a subsequent depletion in the functional Fe compartment, IDE and IDA, begins. Researchers have found that subclinical Fe deficiency could also affect the health of the human body[3]. Subclinical Fe deficiency, when storage Fe has been used up but IDA has not yet developed, can do harm to one's intelligence, memory, health, immunity and work efficiency[4]. But the symptoms in this stage are so subtle that they are quite often neglected and rarely get enough attention. According to one report, the prevalence rate of subclinical Fe deficiency is over two times that of IDA[5].

The lack of a specific sensitive index for screening Fe deficiency as well as inadequate Fe intake from food and the low bioavailability of Fe in food should be held responsible for IDA. Many individuals with subclinical Fe deficiency have not been identified and the problem is not solved in time[6]. As a result, the problem may be aggravated and IDA may develop. This is one of the reasons why the IDA prevalence rate stays at such a high level. Now, a large number of individuals, especially women of child-bearing age, are suffering from subclinical Fe deficiency, so it is critical to find a sensitive, specific and applicable biochemical marker to determine the magnitude of early-stage Fe deficiency. Currently, the concentration of serum transferrin receptor (sTfR) has been reported to be useful in the diagnosis of Fe deficiency[7–9]. It shows a slight increase in the IDs stage and a significant increase in the IDE stage. At this stage its change appears earlier than that of packed cell volume and free erythrocyte protoporphyrin. STfR can also reflect functional Fe status and erythropoietic activity in adults[10–11]. The present study was designed to observe the changes of sTfR level in Fe-deficient women of child-bearing age. We also evaluated the specificity and sensitivity of sTfR in determining Fe deficiency and its diagnostic efficiency for Fe deficiency, and verified whether sTfR could be used as a reliable index for assessing Fe status and determining early-stage Fe deficiency.

Abbreviations: AUC^{ROC}, area under the receiver-operating characteristic curves; IDA, Fe-deficiency anaemia; IDE, Fe-deficiency erythropoiesis; IDs, Fe store depletion; ROC, receiver-operating characteristic; SF, serum ferritin; sTfR, serum transferrin receptor; ZPP, Zn protoporphyrin.

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Subjects and methods

Subjects

The study population originally consisted of 942 women of child-bearing age (18–45 years) who lived in suburban areas of Beijing, and Langfang City of Hebei Province and who were low or middle class. Those with history of treatment with Fe tablets, smoking, drinking, haematological diseases, asthma, musculoskeletal system disease, infection within the past few months, or pregnancy within 1 year before the study were excluded. The protocol of the present study was approved by The Medical Ethics Committee of Peking University. The study was carried out from February to October 2002. Written informed consent was obtained from all participants.

Study design

The Fe status of all subjects was assessed at the very beginning of the study by determining serum ferritin (SF), Zn protoporphyrin (ZPP) and Hb levels in venous blood samples. As suggested by Wang[12], a ferritin cut-off value of 20 μg/l was considered to indicate absent Fe stores and IDs. As suggested by Hastka *et al.*[13], Zn erythropoiesis values >1·4 μg/g Hb and SF <20 μg/l were defined as IDE, while SF <20 μg/l, ZPP values >1·4 μg/g Hb and Hb below 120 g/l were defined as IDA. Subjects were randomly selected from different groups: fifty-six individuals with normal Fe status, fifty-six in the IDs stage, forty-one in the IDE stage and thirty-six in the IDA stage. Then sTfR level was determined and sTfR/SF was calculated (sTfR/logSF and log(sTfR/SF)).

Collection of blood samples and laboratory analysis

Overnight fasting blood specimens (4·0 ml) were obtained by certified laboratory workers for the determination of parameters associated with Fe status. Blood specimens were processed at a local examination centre and shipped to a laboratory in Beijing. A sample of 0·02 ml of whole blood specimen was added to a light-avoided sterile heparin-anticoagulant tube and stored at −4°C. ZPP concentration was determined within 24 h by using an accurate, well-calibrated ZPP haematofluorometer (microfluorescence method, ZPP haematofluorometer model 3800; Guangdong Kangda Development Company, Guangdong, China). A sample of 0·02 ml of whole blood specimen was added to methaemoglobin cyanide dilution to determine Hb level (methaemoglobin cyanide method, model 724 spectrophotometer; Shanghai no. 3 Analysis Instrument Factory, Shanghai, China and model HZ-881K table-top multi-application oven-controlled oscillator; Taicang Scientific Experimental Instrument Factory, Jiangsu, China). The remaining blood was centrifuged to isolate serum, which was stored at −70°C for determination of the SF and sTfR. The SF was determined by the dioimmunological method by using a ^{125}I-serum ferritin kit (The Atomic Energy Institute, Beijing, China, model SN-695B Intelligent Gamma RIA Measurement Instrument; Rui-Huan Instrument Factory of Shanghai Nuclear Research Institute, Shanghai, China and model DDL-5 Freeze Centrifuge; Shanghai AnTing Scientific Instrument Factory, Shanghai, China). The sTfR was determined by an ELISA kit (R&D Systems, Minneapolis, MN, USA and model 450 enzyme calibration enzyme-linked apparatus; Bio-Rad, Hercules, CA, USA).

All members of the study team successfully completed a training programme on the aims of the study and on the specific methods used. The biological measurements were standardised among laboratories according to the criteria of Peking University.

Statistical analysis

Dunnett's *t* test was used to compare the difference in all of the parameters determined between the test groups and normal, and Bonferroni's *t* test was used to compare the difference with the adjacent group with the better Fe status. Univariate correlation was used to analyse the relationship among the variables. Receiver-operating characteristic (ROC) curves were used to compare the corresponding areas of sTfR/logSF, log(sTfR/SF) and sTfR to identify IDs and functional Fe deficiency. All the analyses were conducted with SPSS (version 10.0; SPSS, Inc., Chicago, IL, USA).

Results

Iron status in women of child-bearing age

Table 1 shows that among the subjects of child-bearing age, 65·2 % had normal Fe status, 34·8 % had different kinds of Fe deficiency, 23·4 % were in the IDs stage, 6·7 % were in

Table 1. Iron-related biochemical indices in women of child-bearing-age (*n* 941) of different iron status*
(Mean values and standard deviations)

	Subjects		Serum ferritin (μg/l)		Zn protoporphyrin (μg/g Hb)		Hb (g/l)	
Group	*n*	%	Mean	SD	Mean	SD	Mean	SD
Normal	614	65·2	57·86	35·93	0·77	0·63	139·8	13·7
IDs	220	23·4	12·01†	5·46	0·70	0·30	135·4	14·9
IDE	63	6·7	14·53†	5·59	2·41†	1·32	133·7	10·5
IDA	44	4·7	10·95†	5·96	4·17†	3·45	104·8†	11·8

IDs, Fe store depletion; IDE, Fe-deficiency erythropoiesis; IDA, Fe-deficiency anaemia.
* There are data missing for one woman (original study population consisted of 942 women).
† Mean value was significantly different from that of the normal group ($P<0·05$).

the IDE stage and 4·7 % were in the IDA stage. The ratio decreased along with the aggravation of Fe deficiency.

Serum transferrin receptor and serum transferrin receptor/serum ferritin levels in women of child-bearing age of different iron status

Table 2 shows that in different Fe deficiency stages, sTfR, sTfR/logSF and log(sTfR/SF) increased significantly in progressive Fe deficiency. In the IDs stage, the sTfR increase was relatively slight, while sTfR/SF increased more significantly than sTfR. In the IDE and IDA stages, the increases of sTfR, sTfR/logSF and log(sTfR/SF) were more significant. In different stages, sTfR and sTfR/logSF were significantly different ($P<0·05$).

Correlation between serum transferrin receptor and iron-related biochemical indices

In the IDs stage, there was no significant correlation between sTfR and other Fe-related biochemical indices. When Fe deficiency was aggravated and developed into IDE, significant positive correlation appeared between sTfR and ZPP. The worse the Fe deficiency became, the more significant its correlation with ZPP became. In the IDA stage, sTfR had significant negative correlation with Hb. When statistical analysis combining all the women of child-bearing age of different Fe status was performed, sTfR was also significantly correlated with SF, Hb and ZPP ($P<0·05$), and the correlation coefficients were $-0·332$, $-0·630$ and 0·698, respectively.

Efficiency of serum transferrin receptor and serum transferrin receptor/serum ferritin in determining iron deficiency

The ROC curves of sTfR and sTfR/SF in the identification of Fe deficiency are shown in Fig. 1 and Table 3. The area under the ROC curves (AUC^{ROC}) shows the parameter for distinguishing Fe-deficient women with IDs from healthy ones. The results showed that the most effective index for identifying IDs was log(sTfR/SF), with AUC^{ROC} of 0·990. The second most effective one was sTfR/logSF, with AUC^{ROC} of 0·944, while sTfR was relatively less effective, with AUC^{ROC} of 0·789. The diagnostic efficiency of sTfR and sTfR/log SF in determining functional Fe deficiency was similar; AUC^{ROC} of 0·830 for sTfR, AUC^{ROC} of 0·840 for sTfR/logSF and AUC^{ROC} of 0·833 for log(sTfR/SF).

Discussion

TfR is a kind of transmembrane glycoprotein. It is important for Fe intake of the cell. The expression of TfR on the cell surface is regulated mainly by post-transcriptional regulation of Fe-mediated Fe-reactive element IRE/IRP (Fe-regulatory protein/Fe-responsive element)[14]. When Fe is insufficient in cells, the expression of ferritin decreases and the expression of TfR increases. On the contrary, when Fe overloads, the expression of TfR decreases and the expression of ferritin increases[15]. Therefore, sTfR cellular uptake of Fe and the expression in cells can reflect the body Fe status. In the present study, we found that the mean value of sTfR was 17·97 nmol/l and its 95 % limit was 16·73–19·22 nmol/l when Fe level was normal. sTfR began to increase slightly in the IDs stage, and then increased significantly when Fe deficiency developed into the IDE stage and achieved its highest level in the IDA stage, which indicated that sTfR could reflect the different stages of Fe deficiency. These results are consistent with those of Cook *et al.*, which also indicated that sTfR was a reliable index to reflect early-stage Fe deficiency in tissues[16]. In the present study, sTfR began to increase in the IDs stage, while ZPP began to change in the IDE stage, that is to say, when Fe for Hb synthesis is insufficient, Zn will bind with protoporphyrin instead of Fe to synthesise ZPP. It showed that sTfR was more sensitive to detect Fe deficiency than ZPP. Flowers *et al.* also demonstrated that great changes had been found earlier in the values of sTfR than in other biochemical indices such as free erythrocyte protoporphyrin and packed cell volume for reflecting functional Fe deficiency, which indicated that sTfR was a more sensitive index to reflect functional Fe deficiency[17–18]. Moreover, sTfR values of female subjects in the IDA stage were 2·86 times higher than those of normal controls. Flowers *et al.* also reported that sTfR values of nineteen IDA patients were 3·2 times (which was a higher elevation than the present study) higher than those of normal controls[17]. This might be due to the different severity of Fe deficiency in the subjects. The subjects in the study of Flowers *et al.* were patients in hospital who had very severe anaemia, while the subjects in the present study were selected from a 'normal' population and most of them

Table 2. The changes of serum transferrin (sTfR) in women of child-bearing age of different Fe status (Mean values and standard deviations)

		SF (μg/l)		Zn protoporphyrin (μg/g Hb)		Hb (g/l)		STfR (nmol/l)		sTfR/logSF		Log(sTfR/SF)	
Group	*n*	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Normal	56	45·91	12·43	0·39	0·10	148·4	7·4	17·97	4·65	10·99	3·11	−0·4	0·16
IDs	56	11·40	3·15	0·39	0·10	141·5	8·8	20·69*	5·21	20·94*	8·09	0·27*	0·20
IDE	41	12·62	5·43	2·45	1·38	135·1	10·8	30·09*†	13·9	33·11*†	23·50	0·39*	0·29
IDA	36	11·60	5·88	4·37	3·56	103·9	12·2	49·85*†	30·4	52·14*†	42·70	0·64*†	0·42

SF, serum ferritin; IDs, Fe store depletion; IDE, Fe-deficiency erythropoiesis; IDA, Fe-deficiency anaemia.
* Mean value was significantly different from that of the normal group ($P<0·05$).
† Mean value was significantly different from that of the adjacent group with the better Fe status ($P<0·05$).

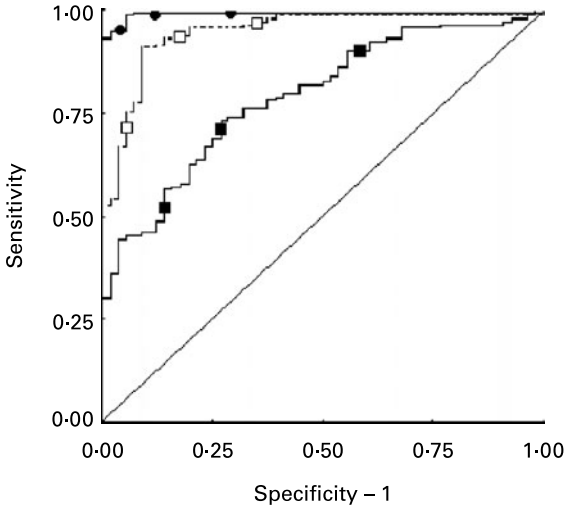

Fig. 1. Receiver-operating characteristic curve of using serum transferrin receptor (sTfR; –■–) and sTfR/serum ferritin (SF) in determining storage Fe deficiency. (—), Reference line; (–●–), log(sTfR/SF); (- □ -), sTfR/logSF.

had mild anaemia. This might be one of the causes for the sTfR differences. In animal experiments, the sTfR levels in rat models with severe IDA were several times higher than those in normal controls[18–19].

Different Fe-related biochemical indices can reflect the stages and severities of Fe deficiency. That SF begins to decrease means the body is in storage Fe-deficiency stage. When functional Fe is insufficient, ZPP increases, which can indicate the stage and severity of functional Fe deficiency[13]. Hb reflects the severity of Fe deficiency. When Hb concentration is lower than the normal level, there will be some additional physiological phenomena associated with anaemia. The present study analysed the correlation between sTfR and other Fe-related biochemical indices in women of different Fe status. We found that the correlation was not significant in the IDs stage. However, sTfR showed significant positive correlation with ZPP in the IDE stage, and this correlation got stronger along with the development of Fe deficiency and reached its highest level in the IDA stage. This indicated that sTfR could serve as a sensitive index for evaluating the functional Fe status. In the IDA stage, sTfR showed significant negative correlation with Hb, which indicated that sTfR could reflect the severity of Fe deficiency. After statistical analysis combining all the women of child-bearing age of different Fe status was performed, we found that sTfR still had significant correlation with other indices, for example, sTfR had positive correlation with ZPP, and it had significant negative correlation with SF and Hb. This indicated that sTfR could not only reflect the body storage Fe and functional Fe status, but can also be used to determine the severity of Fe deficiency. sTfR itself could be used to evaluate body Fe status. Some studies showed that its sensitivity would improve if sTfR and SF were combined in evaluating body Fe status.

The present study showed that sTfR/SF, i.e. sTfR/logSF and log(sTfR/SF), could sensitively reflect the changes from normal Fe status to storage Fe deficiency in the IDs stage. Log(sTfR/SF) had the most significant change in the IDs stage, so it had the highest sensitivity in determining the IDs stage. Punnonen *et al.* and Malope *et al.* also thought that sTfR/logSF could serve as a reliable index for the IDs stage[20,21]. Malope *et al.* found that log(sTfR/SF) could differentiate the stages of Fe deficiency more clearly[21]. This is because the expression of cellular TfR and ferritin is regulated by Fe-mediated IRE/IRP (Fe-regulatory protein/Fe-responsive element). When Fe is insufficient, the expression of TfR increases and that of SF decreases, so the ratio significantly changes. When functional Fe is insufficient, the change of sTfR/SF is similar to that of sTfR. This is because that SF stays consistently at a lower level in this stage and sTfR/SF does not change significantly. According to these results, log(sTfR/SF) could enhance the efficiency of sTfR in diagnosis of storage Fe deficiency, but could not increase the sensitivity of sTfR in diagnosis of functional Fe deficiency. So detection of sTfR could be used to diagnose functional Fe deficiency effectively. Using sTfR to diagnose Fe deficiency has important significance in improving the Fe status in the population.

The use of ROC curves is an effective method to evaluate diagnostic tests comprehensively and precisely. The method can be used to identify the diagnostic efficiency of tests by calculating AUC^{ROC}. It takes sensitivity (true positive rate) as the *y*-coordinate and 1 – specificity (false positive rate) as the *x*-coordinate. Many pairs of true positive rates and false positive rates in the correlation comprise the whole curve. Values of AUC^{ROC} range between 1·0 (perfect separation of the values of two groups) and 0·5 (no apparent distributional difference between two groups of the values). This is a quantitative, descriptive expression of how close the AUC^{ROC} is to the perfect one (value = 1·0)[22]. The ROC curve was used to analyse the diagnostic efficiency of sTfR and sTfR/SF. The comparison of areas under ROC curve of sTfR and sTfR/SF in determining storage Fe deficiency indicated that log(sTfR/SF) had the highest efficiency in determining storage Fe deficiency, which reached 99 %. The best point of tangency was 0·047, when sensitivity was 93 % while specificity was 100 %, and the positive expected value was 91 % while the negative expected value was 100 %. When we used a ROC

Table 3. The sensitivity and specificity of using serum transferrin (sTfR) and sTfR/serum ferritin (SF) in determining storage iron deficiency and functional iron deficiency (iron deficiency erythropoiesis and iron deficiency anaemia)

	In determining storage Fe deficiency			In determining functional Fe deficiency		
	Efficiency	Sensitivity of cut-off point (%)	Specificity of cut-off point (%)	Efficiency	Sensitivity of cut-off point (%)	Specificity of cut-off point (%)
sTfR	0·789	75	68	0·830	79	63
Log(sTfR/SF)	0·990	91	100	0·840	75	65
sTfR/logSF	0·944	91	90	0·833	81	60

curve to analyse the efficiency of several indices in determining functional Fe deficiency, it showed that they had similar diagnostic efficiency. The efficiency in diagnosis of functional Fe deficiency did not increase when sTfR and SF were combined. So, a single test of sTfR was a good index for determining functional Fe deficiency.

The present study showed that both sTfR and sTfR/SF could specifically reflect the severity of body Fe deficiency, and they could serve as reliable indices for evaluating Fe status in childbearing-aged women and determining Fe deficiency.

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