Malaysian Journal of Medicine and Health Sciences (ISSN 1675-8544); Vol. 9 (2) June 2013: 3-12

Serum Soluble Transferrin Receptor Concentration as a Biomarker of Erythropoietic Activity: Surrogate Marker of Adequate Transfusion in Adult Beta-Thalassaemia Intermedia Patients

[1]S Thambiah*, [1]E George, [2]U Nor Aini, [3]J Sathar, [1]H Zarida & [2]Mokhtar AB

[1]Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor Darul Ehsan, Malaysia

[2]Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia

[3]Department of Haematology, Hospital Ampang, Jalan Mewah Utara, Taman Pandan Mewah, 68000 Ampang, Selangor Darul Ehsan, Malaysia

ABSTRACT

Management of Beta (β)-thalassaemia intermedia in contrast to β-thalassaemia major patients has no clear guidelines as to indicators of adequate transfusion. Regular blood transfusion suppresses bone marrow erythropoietic activity. Serum soluble transferrin receptor (sTfR) concentration is a marker for erythropoietic activity, with increased sTfR being associated with functional iron deficiency and increased erythropoietic activity. This study aimed to determine the use of sTfR as an indicator of adequate transfusion in adult β-thalassaemia intermedia patients. A cross-sectional study was conducted at Hospital Ampang, Malaysia, for six months. Patient group included six β-thalassaemia intermedia and 34 HbE-β-thalassaemia transfused patients. None of the patients were on regular monthly blood transfusions as in β-thalassaemia major. The control group comprised of 16 healthy subjects with normal haematological parameters. Haemoglobin (Hb) analysis, sTfR and ferritin assays were performed. Hb and HbA percentages (%) were found to be significantly lower in patients compared to the controls, while HbE%, HbF%, sTfR and ferritin were significantly higher in patients. An inverse relationship was found in the controls between HbF% with Hb ($r = -0.515$, $p < 0.05$) and HbA% ($r = -0.534$, $p < 0.05$). In patients, sTfR showed an inverse relationship with HbA% ($r = -0.618$, $p = 0.000$) and a positive correlation with HbE% ($r = 0.418$, $p = 0.007$) and HbF% ($r = 0.469$, $p = 0.002$). Multivariate analysis showed that HbA% ($r = 2.875$, $p = 0.048$), HbE% ($r = 2.872$, $p = 0.020$) and HbF% ($r = 2.436$, $p = 0.013$) best predicted sTfR independently in patients. Thus, sTfR is a useful marker for erythropoiesis. The elevated sTfR in these patients indicate that the transfusion regimen used was inadequate to suppress ineffective erythropoiesis. Hb levels may not be the best target for monitoring transfusion treatment in β-thalassaemia intermedia patients, but the use of sTfR is helpful in individualising transfusion regimens.

Keywords: Serum soluble transferrin receptor, serum ferritin, β-thalassaemia intermedia/HbE-β-thalassaemia, ineffective erythropoiesis

INTRODUCTION

Beta-thalassaemia intermedia, a clinical condition between thalassaemia minor and thalassaemia major, ranges from mild anaemia without bone abnormalities to severe anaemia with haemoglobin (Hb) less or equal to 7.0 g/dL, with increased erythropoietic activity[1]. In Asia, HbE-β-thalassaemia remains one of the most common haemoglobinopathies[1, 2].

Iron overload remains the major cause of morbidity in thalassaemia patients regardless of their blood transfusion history. This is because of the significant increase in gastrointestinal iron absorption to supply the overwhelming production of ineffective erythropoiesis[3]. Nonetheless, no physiological mechanism exists for eliminating excess iron from the body[4]. Electron transfer capacity of ionic forms of iron leads to formation of highly reactive hydroxyl radicals, which damage lipids, proteins and DNA molecules leading to cell death[5]. Approximately 2000 to 4000 thalassaemia patients die each year from iron overload worldwide because chelation therapy is expensive and cumbersome to administer[6].

The transferrin receptor (TfR) is a 760-amino-acid glycoprotein found on all cell surfaces except mature erythrocytes; 80% of which is in the erythroid marrow. The total mass of cellular TfR depends on both the number of

*Corresponding author: *subashini@upm.edu.my*

erythroid precursors in the bone marrow and on the number of TfR per cell, a function of iron status of the cell. Thus, excess iron suppresses TfR synthesis.[7] Patients with secondary iron overload and hereditary haemochromatosis have been shown to have decreased TfR expression in their cells[8]. There are two different transferrin receptors, namely, transferrin receptor 1 (TfR1) and transferrin receptor 2 (TfR2)[9]. In this paper, TfR alludes to TfR1.

Serum soluble transferrin receptor (sTfR) is a soluble truncated monomer of TfR, lacking its first 100 amino acids, which circulate in the form of a complex of transferrin and its receptor[7]. sTfR shows no correlation with gender or age and has low between-day intra-individual variation and overall biological variation[9]. Meanwhile, sTfR concentration has been shown to be proportional to the mass of erythroid tissue by conventional ferrokinetic studies; hence, erythropoietic activity has been found to be the most important determinant of sTfR levels[10]. In conditions of erythroid hypoplasia, for example in hypertransfusion, chronic renal failure, severe aplastic anaemia, or after intensive chemotherapy, sTfR concentrations are decreased whereas its levels are increased in stimulated erythropoiesis such as congenital dyserythropoietic anaemia, haemolytic anaemia, hereditary spherocytosis, sickle cell anaemia, thalassaemia major or intermedia, megaloblastic anaemia or secondary polycythaemia. Serum sTfR concentration ranges from approximately 0.5 mg/L, contributed mainly by non-erythroid tissues when erythropoiesis is totally absent to about 100 mg/L in severely anaemic thalassaemia patients[7, 11].

Based on the current understanding of the regulation of TfR expression at the cellular level and the fact that sTfR measurements have low biological and analytical variations[9], this study was designed to determine the serum sTfR concentration in transfused β-thalassaemia intermedia/HbE-β-thalassaemia adult patients as a quantitative measure of erythropoietic activity.

MATERIALS AND METHODS

A total of 40 adult transfused β-thalassaemia intermedia/HbE-β-thalassaemia patients, who were regularly followed up at the Haematology specialist outpatient clinic at Hospital Ampang, were recruited for the study. Inclusion criteria were based on the following at the time of diagnosis: (i) Clinical diagnosis: clinical severity of the disease is greater than the mild symptoms of β-thalassaemia trait and less than the severe manifestations of β-thalassaemia major; (ii) Patient maintains a Hb level of 7 to 10g/dL, and (iii) Patient ***does not*** require regular blood transfusion at intervals of 2 to 4 weeks as in β-thalassaemia major[5].

Meanwhile, the exclusion criteria comprised of patients with β-thalassaemia intermedia less than 15 years old, fever or any signs or symptoms of infection at the time of blood taking and a history of transfusion reaction. Subjects eligible for the study were identified by reviewing their medical records prior to the clinic appointment. All details of the transfusion or chelation programme were extracted from their hospital records. During the clinic visit, each subject was invited to participate in the study after reading the information sheet on the research project and following a verbal explanation by the recruiting researcher. An informed written consent was also obtained from each subject who participated in this study. The study was approved by the ethical review boards of the institutions involved.

The control group comprised of 20 subjects; only 16 were chosen and four (all females) were excluded due to iron deficiency anaemia. Iron deficiency anaemia was diagnosed based on serum ferritin and sTfR values of less than 10 µg/L and 4.4 mg/L, respectively. The control group was not matched for age and gender, as no correlation was found between sTfR and these variables in previous studies[9].

On the morning of the clinic visit, a maximum of 10 mls of venous blood was collected from each patient into plain and ethylenediaminetetra-acetic acid (EDTA) tubes. The blood samples from the plain tubes were centrifuged and the serum was aliquoted and frozen at -20°C until analyses of serum ferritin and sTfR levels were carried out. Measurements of serum ferritin and sTfR levels were done in the chemical pathology laboratory of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) not later than one month after the sample collection for optimum recovery and in batches to minimise analytical variation. The blood samples in EDTA tubes were sent to the haematology laboratory of Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM) for Hb, red blood cell counts, red cell indices and Hb analysis to confirm β-thalassaemia intermedia/HbE-β-thalassaemia status. Similarly, blood samples for the control group were analysed for serum ferritin, sTfR and Hb analysis.

Serum sTfR was measured by particle enhanced immunoturbidmetric method on Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany). The reference range is 2.2 – 5.0 mg/L for males aged 18 to 60 years and 1.9 – 4.4 mg/L for females aged 18 to 45 years. The stability of serum sTfR is as follows: 3 days at 20 – 25°C; 7 days at 4 – 8°C and 4 weeks at –20°C. Serum ferritin was measured using Chemiluminescent Microparticle Immunoassay (CMIA) technology on Architect System (Abbott Laboratories, Abbott Park, United States of America). The reference range is 21.8 – 274.7 µg/L for males and 4.6 – 204.0 µg/L for females, respectively.

Hb, red blood cell counts and red cell indices were measured on an automated blood counter (Coulter STKS, Coulter Corporation, 11800 SW, 147, Miami, Florida 33196-2500, United States of America). Hb analysis was performed on VARIANT (Bio-Rad, 2000 Alfred Nobel Dr., Hercules, CA 94547, United States of America), a fully automated high performance liquid chromatography (HPLC) system that uses double-wave length detection (416 and 690 nm). This cationic-exchange column chromatography enables qualitative determinants of HbA_2, HbF and abnormal haemoglobins in 6.5 minutes. In the β-thalassaemia short programme (BTS), a 3 X 4.6 cm non-porous cationic exchange column is eluted at a flow rate of 2 ml/min by a gradient created by two phosphate buffers that differ in pH and ionic strength. On this system, HbE co-elutes with HbA_2 with the same retention time. The values of HbA_2 of more than 10% at this retention time is considered as Hb variant (mean HbA_2 + HbE). In Malaysia, the reference range for HbA_2 in normal subjects using VARIANT is 2.1 – 3.3%.

Data were analysed and reported using the Statistical Package for Social Sciences (SPSS Version 18) statistical software. Shapiro-Wilk, instead of Kolmogrov-Smirnov test, was used to assess sample distribution due to the small sample size (less than 100). Skewness and kurtosis values were also used to assess normality of data. Results were expressed as mean values and standard deviation for normally distributed variables. Non-parametric tests were used for analysis of variables, which were not normally distributed, and median values with 25 to 75 percentiles were used. The Student's T-test and/or the F-test (analysis of variance) were used to test the probability of any significant difference between groups for the normally distributed data, whereas the Mann-Whitney U test and/or Kruskall Wallis test were used for the non-normal distribution of variables. Pearson's or Spearman's correlation coefficients were calculated to estimate any linear correlation between continuous variables as appropriate. Linear regression analysis was employed to explore the association between sTfR and the following parameters: Hb, HbA%, HbA_2/HbE%, HbF%, and ferritin. In all the statistical analyses, p value of < 0.05 (95% confidence interval) is considered to be statistically significant.

RESULTS

The demographic data and characteristics of the patients and control groups are shown in Table 1. There was no statistical difference between the two groups with regards to gender and race, while median age was significantly higher in the patient group compared to that of the control group. All other data were not applicable to the control group. The patient group consisted of 34 HbE-β-thalassaemia and six β-thalassaemia intermedia. Nineteen (47.5%) of them had undergone splenectomy, 17 (42.5%) had been transfused only once a year, and 15 (37.5%) were not on any chelation therapy.

Table 1. Demographic and clinical details of control and patient groups

	Control group (N = 16)	Patient group (N = 40)	Statistical test	p value*
Age (years)				
Range	20 - 35	16 - 49	Z = -3.042	0.002
Median	(21.0)	(28.5)	(Mann-Whitney U test)	
Sex				
Male	9 (56.2%)	14 (35%)	$\chi^2 = 2.132$	0.144
Female	7 (43.8%)	26 (65%)	(Pearson Chi-square)	
Race				
Malay	11 (68.8%)	32 (80%)	$\chi^2 = 0.811$	0.368
Non-Malays	5 (31.2%)	8 (20%)	(Pearson Chi-square)	
Patient status	Not applicable			
HbE-β-thalassaemia		34 (85%)		
β-thalassaemia intermedia		6 (15%)		
Splenectomy	Not applicable			
Yes		19 (47.5%)		
No		21 (52.5%)		
Transfusion frequency (times/per year)	Not applicable			

Continued

Table 1. *Continued*

	Control group (N = 16)	Patient group (N = 40)	Statistical test	p value*
1		17(42.5%)		
2		4 (10%)		
3		5 (12.5%)		
4		4 (10%)		
5		4 (10%)		
6		2 (5%)		
7		1 (2.5%)		
8		-		
9		1 (2.5%)		
10		-		
11		-		
12		2 (5%)		
Chelation therapy	Not applicable			
- Desferral compliant		1 (2.5%)		
- Desferral non-compliant		1 (2.5%)		
- Ferriprox compliant		14 (35%)		
- Desferral and Ferripox compliant		4 (10%)		
- Desferral and Ferriprox non-compliant		5 (12.5%)		
- Not on chelation therapy		15 (37.5%)		

*Statistical significance at $p < 0.05$

Table 2 represents the laboratory data of the patient and control groups, expressed as mean ± SD. The difference was shown to be significant for all the numerical parameters between the two groups. Both Hb and HbA% were significantly lower in the patient group compared to the control group, whereas HbA_2/HbE%, HbF%, sTfR and ferritin were significantly higher in the patient group. In the control group, the only significant correlation found was the inverse relationship between HbF% with both Hb and HbA% In the patient group, no significant correlation was found between Hb and all other numerical parameters. The significant correlations in the patient group are summarised in Table 3. In the multivariate linear regression analysis presented in Table 4, HbA%, HbA_2/HbE%

Table 2. Laboratory data of control and patient groups

	Control group (N = 16) min-max (mean ± SD) or (median)	Patient group (N = 40) min-max (mean ± SD) or (median)	Statistical test	p value*
Hb (g/dL)	10.6 – 16.3 (13.9)	3.7 – 9.7 (7.7)	$Z = - 5.806^a$	$p < 0.0005$
HbA_0 (%)	84.6 – 89.2 (87.6)	1.4 – 84.4 (25.2)	$Z = - 5.805^a$	$p < 0.0005$
HbA_2/HbE (1 – 3.5%)	2.4 – 5.8 (2.8)	3.7 – 78.3 (37.4)	$Z = - 5.715^a$	$p < 0.0005$
HbF (< 1%)	0 – 0.8 (0.2 ± 0.2)	0.4 – 69.4 (26.6 ± 18.9)	$t = 8.833^b$	$p < 0.0005$
sTfR (mg/L)	1.2 – 4.7 (3.1 ± 0.9)	6.1 – 45.1 (23.1 ± 10.2)	$t = 12.315^b$	$p < 0.0005$
serum ferritin (µg/L)	11.6 – 337.5 (55.4)	214.5 – 18340.3 (2870.6)	$Z = - 5.713^a$	$p < 0.0005$

a - Mann-Whitney U test; b – Student's T-test
* Statistical significance at $p < 0.05$

Table 3. Significant correlations found between parameters in the patient group

Parameter	HbA_0%	HbA_2/HbE%	HbF%	Ferritin
sTfR	r = - 0.618 p = 0.000	r = 0.418 p = 0.007	r = 0.469 p = 0.002	-
HbA_0%	-	r = - 0.767 p = 0.000	r = - 0.574 p = 0.00	r = 0.436 p = 0.005
Ferritin	-	-	r = - 0.384 p = 0.014	-

* Statistical significance at $p < 0.05$

Table 4. Linear regression analysis of stfr as the dependent and Hb, HbA0%, HbA2/HbE%, HbF%, and ferritin as independent variables in patient group

Variables	β coefficients	t value	p value*
Hb	0.097	0.097	0.512
HbA_0%	**2.875**	**2.052**	**0.048**
HbA_2%/HbE%	**2.872**	**2.436**	**0.020**
HbF%	**2.436**	**2.623**	**0.013**
Ferritin	0.266	1.667	0.105

* Statistical significance at $p < 0.05$
The significant variables are shown in bold.
$R^2 = 0.502$

and HbF% best predicted the value of sTfR in β-thalassaemia intermedia/HbE-β-thalassaemia patients. However, mean Hb and serum ferritin could not independently predict the sTfR in these patients.

DISCUSSION

Pootrakul *et al*. (1988) have shown that in HbE-β-thalassaemia patients, progressive iron loading is a result of increased amount of iron absorbed exceeding that which is lost. This occurs when erythroid activity increases to over five times normal with a high degree of ineffective erythropoiesis. However, this increased iron absorption can be reversed if red cell turnover is normalised by adequate transfusion or marrow transplantation as it will suppress both the red cell production and the increased red cell destruction, as defective cells are replaced by normal cells. Plasma sTfR concentration reflects the degree of erythropoiesis; being increased in the states of enhanced erythropoiesis and vice versa[12].

Circulating sTfR also reflects the body's iron status. sTfR has been reported to increase in the presence of iron-deficiency anaemia, but it remains unclear whether it is decreased with iron overload states[8]. Ledue and Craig (1995) observed low concentrations of sTfR in subjects with hereditary haemochromatosis, whereas Huebers *et al*. (1990) and Baynes *et al*. (1994) reported normal sTfR concentrations in patients with idiopathic haemochromatosis and hereditary haemochromatosis, respectively. Thorstensen and Romslo (1992) found low sTfR concentrations in males with increased transferrin saturations while Centis *et al.* (1995) found a significant negative correlation between sTfR concentrations and ferritin concentrations in post-bone marrow transplanted thalassaemia patients.

Using the particle enhanced immunoturbidimetric method in this study, the sTfR levels averaged 3.1 ± 0.9 mg/L in the control group (n = 16), which is within the reference range given by the manufacturer. Beguin (2003) reported a mean sTfR level of 5.0 ± 1.0 mg/L in normal human subjects, which remained stable over time in the same subjects. This disparity in the sTfR values in various commercial assays is due to lack of an international standard. Therefore, although there are some correlations of sTfR levels between these different assays, a direct clinical comparison of these differing values obtained may be difficult[7].

In this study, we looked at the sTfR in assessing iron status and as an indicator of adequate suppression of erythropoiesis with adequate transfusion. sTfR values in the patient group were significantly higher compared to the control group. If we consider the transfusion regime sufficient, a reasonable hypothesis for the raised sTfR could

be that a functional deficit in iron availability for erythropoiesis exists, giving rise to increased iron absorption and iron overload state. This could occur in thalassaemic patients with co-existing anaemia of chronic disease. Although sTfR levels are known to be normal in anaemia of chronic disease, it may be raised in patients with both thalassaemia and chronic diseases. In anaemia of chronic disease, disturbance in iron homeostasis is due to increased uptake and storage of iron within reticuloendothelial cells, leading to subsequent limitation of iron availability for erythroid progenitor cells and iron-restricted erythropoiesis[18].

Another possibility could be that in thalassaemia, iron content of circulating transferrin could be altered, thus reducing the affinity of the transferrin receptor (TfR) for its ligand, and iron uptake into the cell. Similarly, inappropriate pH level within the endosomes may also contribute to the unavailability of iron to the cell[19, 20].

Co-inheritance of haemochromatosis protein (HFE) mutations has a substantial role in iron overload in β-thalassaemia carriers in north European populations, where two HFE mutations (C282Y and H63D) are prevalent. Sharma *et al*. (2007) concluded that thalassaemia intermedia patients, with co-existent HFE mutation H63D, have a higher likelihood of developing iron overload, irrespective of blood transfusions and may require early iron chelation. However, a study on HbE-β-thalassaemia individuals reported that because of the paucity of HFE mutations in Thailand, it appears that these alleles are less likely to be responsible for high ferritin levels and iron loading in these patients[22].

In the context of our current understanding of HFE/TfR interactions, undiscovered mutation/s of HFE, pH variability or TfR:HFE stochiometry discrepancy could disrupt iron homeostasis in thalassaemia[19, 23, 24]. A working hypothesis by Cazzola *et al*. (1999) is that high sTfR levels may alter the ratio between HFE and TfR, and thus inactivating the inhibitory effect of HFE protein on intestinal iron absorption. This supports the observation that iron absorption is increased both in iron deficiency and in iron-loading anaemias, the two contrasting conditions whose only common denominator is increased sTfR[25]. If population studies indicate greater prevalence of HFE mutations in thalassaemia patients, it is necessary to screen HFE gene mutations[26]. In Malaysia, however, the prevalence of HFE mutations is not known.

While sTfR has been proven to be a reliable marker of tissue iron deficiency, the interpretation of an individual value may be difficult in a patient in whom both changes in erythropoietic activity and iron status occur simultaneously[7] in patients with β-thalassaemia intermedia, the iron status being affected by both increased iron absorption via gastrointestinal tract and from blood transfusion. A study by Cazzola *et al*. (1995) indicated that erythroid activity in β-thalassaemia major patients, measured using sTfR, is 1 to 2 times normal for pre-transfusion Hb levels between 10 and 11g/dl, 1 to 4 times normal for Hb levels from 9 to 10 g/dL, and 2 to 6 times normal for Hb levels from 8.5 to 9 g/dL. Although the sTfR levels in the patient group was about 7 times higher than in the control group for mean Hb of 7.5 g/dL (range 3.7 – 9.7), no clear relationship was observed between mean Hb and sTfR in this study. The considerable variation in the Hb range in these patients may reflect the wide range of transfusion regimens used.

The mean Hb concentration and the mean HbA% were significantly lower in the patient group compared to the control group, whereas the mean HbA_2/HbE% and mean HbF% were significantly higher in the patient group compared to the control group. These findings are consistent with the laboratory diagnosis of β-thalassaemia, whereby low Hb correlates with the anaemia in these patients and the high HbA_2/HbE% and HbF% and low HbA% are consistent with the Hb subtype pattern, revealing the absence or complete absence of HbA with almost all the circulating haemoglobin being HbF and normal, low or slightly raised HbA_2/HbE[4]. This pattern also explains the inverse relationship between HbA% with HbF% ($r = -0.574$, $p = 0.000$) and HbA_2/HbE% ($r = -0.767$, $p = 0.000$), respectively in the patient group.

Foetal haemoglobin [HbF ($\alpha_2\ \gamma_2$)] is approximately less than 1% in normal, healthy adults and it is confined to a subset of red blood cells called F cells, which constitute about 3% of the erythrocytes.[28] In β-thalassaemia, the production of HbF ($\alpha_2\gamma_2$) helps to *mop up* excessive α-chains that precipitate in red blood cells and causes ineffective erythropoiesis and haemolysis.[4] Data from a study by Rees *et al*. (1999) suggested that the high HbF% in HbE-β-thalassaemia, and other β-thalassaemia syndromes, resulted from increased erythropoietin levels to bone marrow expansion, and possible increased F-cell production, combined with ineffective erythropoiesis, to give a survival advantage to F cells. sTfR has been shown to correlate with the mass of erythroid tissue by ferrokinetic studies[7, 29]. Therefore, the positive correlation between sTfR and HbF% ($r = 0.469$, $p < 0.01$) found in this study shows evidence of greater degree of ineffective erythropoiesis and possibly F-cell selection in β-thalassaemia patients.

Patients with β-thalassaemia do not produce enough HbA ($\alpha_2\beta_2$) because their cells have reduced or the absent synthesis of β-polypeptide chain of human haemoglobin[30]. As such, the inverse correlation between HbA% and sTfR in the patient group ($r = -0.618$, $p < 0.01$) points to an inadequate or ineffective transfusion regimen, whereby HbA% is the transfused blood and the high sTfR values reflect markedly enhanced ineffective erythropoiesis. This relationship suggests that the transfusion given to these patients is insufficient to suppress ineffective erythropoiesis.

The optimal transfusion regimen remains a contradictory matter. In the early days, thalassaemia patients were transfused at Hb of 6 to 7 g/dl when they presented with symptomatic anaemia. However, these patients ended up with severe bone deformities due to bone marrow expansion and enlarged liver and spleen, both secondary to intense erythropoiesis. Consequently, a transfusion regimen to maintain pre-transfusional Hb at 10 to 12 g/dl was adopted to completely suppress bone marrow expansion. Unfortunately, these patients with higher baseline Hb required more blood volume and therefore became iron-overloaded. Thus, a pre-transfusion Hb of 9 to 10 g/dL, at which the erythroid marrow activity is two to three times normal, has been adopted by many institutions managing β-thalassaemia intermedia patients to decrease iron absorption through erythroid marrow suppression[31].

Although sTfR levels have demonstrated that regular-interval blood transfusions have better marrow suppression than sporadic transfusions, it is noted that in some patients who are either overtransfused or there is insufficient inhibition of ineffective erythropoiesis, sTfR measurement is inadequate. Thus, the assay of sTfR may be more useful to individualise the transfusion regimen[7].

In the patient group, the serum ferritin was found to have a positive relationship with HbA% ($r = 0.436$, $p = 0.005$), whereby the HbA comes from blood transfusion and raised serum ferritin indicates iron overload from transfused blood. However, the inverse correlation between serum ferritin and HbF% ($r = -0.384$, $p = 0.014$) suggests the existence of some suppressions of ineffective erythropoiesis by blood transfusion although inadequate, as indicated by the raised sTfR levels.

On the basis of current understanding of regulation of TfR expression at the cellular level, the general hypothesis is that sTfR concentrations would be reduced in iron overload states. In this study, however, the patients' sTfR and ferritin levels were significantly raised. An inverse correlation between sTfR and ferritin found in a study of healthy term infants was explained to represent an association between erythropoietic activity and iron utilisation for erythropoiesis rather than an effect of iron stores on TfR expression[7]. Therefore, sTfR may only be considered as a marker of erythropoiesis when there is adequate iron stores. Additionally, sTfR may become a marker of iron status when tissue iron deficiency occurs, with or without adequate iron stores[7].

Iron deficiency was excluded in these patients with high sTfR by using serum ferritin, which is an indirect estimate of body iron stores. Although serum ferritin (i.e. an acute phase protein) is known to increase in certain pathological states, it is routinely used in most hospitals as a measure of iron status and also in monitoring of chelation therapy due to its practical and non-invasive feature. In these patients, infection was excluded primarily on clinical grounds, which included being afebrile and in generally an asymptomatic state.

Slight decreases in sTfR and sTfR: ferritin have been reported in conditions with iron overload but conflicting results have been obtained[9]. Previous studies have shown that within the iron-replete range, sTfR correlates with Hb but not with markers of iron status such as transferrin saturation and ferritin[7]. In this study, there was no significant correlation found between sTfR and serum ferritin in the patient group, although both values were significantly raised compared to the control group. This raised sTfR is due to the fact that erythropoietic progenitor cells in bone marrow are increased as a measure for compensation of anaemia. This leads to an over-expression of TfR, and as a result of the increased iron absorption or transfusions possibly also to an iron overload state, it markedly raised the ferritin levels. In these thalassaemic patients, increased sTfR does not indicate an increased iron need but only reflects increased erythropoietic activity. As such, the high sTfR levels in these patients, once again, points to inappropriate transfusion regimen.

Previous studies have shown that sTfR provides a measure of the efficiency of erythropoiesis when used in combination with an absolute reticulocyte production index. The relative increase in the absolute reticulocyte production index seen in haemolytic anaemias with efficient erythropoiesis (autoimmune haemolytic anaemia, sickle cell anaemia, and hereditary spherocytosis) is a measure of effective erythropoiesis and closely parallels the increase in sTfR. Alternatively, although sTfR is markedly increased in red cell disorders with ineffective erythropoiesis (thalassaemia, megaloblastic anaemia, and the myelodysplastic syndromes), the reticulocyte production index remains minimally changed. Thus, a greatly increased sTfR, with a normal reticulocyte production index, defines ineffective erythropoiesis, provided that iron deficiency is excluded[32]. However, in this study, absolute reticulocyte production index was not included.

Nonetheless, a few issues of concern are overlooked in this study. sTfR is affected by different confounding variables, such as abnormal renal function or vitamin B12 and folic acid deficiency, were not considered in this study[33]. Furthermore, a marked increase in sTfR among β-thalassaemia intermedia/HbE-β-thalassaemia patients, which may be associated with the severity of the clinical manifestations of this disease, was also not stratified in this study[2]. Also, with regards to the transfusion regimen, only the number of transfusions per year was taken into account and not the inter-transfusion duration or the actual transfusion regimen with regards to the amount of blood transfused (ml/kg/day) and iron calculation per transfusion. Thus, future studies could take these factors into consideration.

Despite these limitations, sTfR appeared to reflect the erythropoietic activity in these patients when correlated with HbF% ($r = 0.469$, $p = 0.002$), and the linear regression analysis showed that HbA% ($\beta = 2.875$, $p = 0.048$), HbA_2/HbE% ($\beta = 2.872$, $p = 0.020$) and HbF% ($\beta = 2.436$, $p = 0.013$) are independent predictors of sTfR. The study by Cazzola *et al*. (1995) suggest that sTfR is a reliable indicator of erythroid suppression in β-thalassaemia major and that a transfusion programme with a baseline haemoglobin of 9 to 10g/dL may provide enough suppression of erythropoiesis in β-thalassaemia major and allow a significant reduction in blood consumption when compared with the classic hyper- or supertransfusion schemes. In Hospital Ampang, patients with Hb levels less than 8g/dL were to be transfused. The mean pre-transfusion Hb in this study was 7.5 g/dL.

CONCLUSION

This study has shown that sTfR is a useful marker for erythropoiesis. The elevated sTfR levels seen in β-thalassaemia intermedia/HbE-β-thalassaemia patients in this study indicated that the transfusion regimen used was inadequate to suppress ineffective erythropoiesis. Therefore, the present transfusion/chelation programme in β-thalassaemia intermedia/HbE-β-thalassaemia patients of Hospital Ampang is inadequate in achieving effective erythroid marrow suppression, and thus, preventing long-term complications. Also, since fixed Hb levels may not be the best target for transfusion treatment in all thalassaemic patients, the use of sTfR may be helpful in individualising transfusion regimens[28]. Further research needs to be done to elucidate the existence of any functional deficit in iron availability to erythroid cells in β-thalassaemia intermedia. These studies should also include HFE/TfR interactions and HFE gene mutations.

ACKNOWLEDGEMENTS

This project was funded by a grant through the Research Fund of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) (FF-020-2007). The authors would like to extend their gratitude to both Associate Professor Dr. Syed Zulkifli Syed Zakaria of UKMMC and Professor Dato' Dr. Lye Munn Sann of Universiti Putra Malaysia (UPM) for their contributions in the statistical analysis. Acknowledgement is also extended to thank all staff of the Haematology Unit, Hospital Ampang, especially the staff nurse Dayang Sumarni bt Abdul Aziz of the thalassaemia clinic, Encik Hamzah Mohamad Yunus, a senior product executive of Roche Diagnostics (M) Sdn. Bhd. and the laboratory staff of UKMMC and Faculty of Medicine and Health Sciences, UPM for their assistance in this project.

REFERENCES

[1] Borgna-Pignatti C. Modern Treatment of Thalassaemia Intermedia. British Journal of Haematology 2007; 138: 291-304.

[2] Boonchalermvichian C, Paritpokee N, Bhokaisawan N, Nuchprayoon I, Wiwanitkit V. Marked increase in serum transferrin receptor among Thai children with HbE-β Thalassaemia. Journal of Paediatric Child Health 2002; 38: 601-3.

[3] Weatherall DJ, Clegg JB. The Thalassaemia Syndromes (4th Ed.). Oxford: Blackwell Science Ltd. 2001.

[4] Hoffbrand AV, Pettit JE, Moss PAH. Essential Haematology (4th Ed.). Oxford: Blackwell Science Ltd. 2003.

[5] Porter J. Pathophysiology of Iron Overload. Hematology/Oncology Clinics 2005; 1: 7-12.

[6] Beutler E, Hoffbrand AV, Cook JD. Iron Deficiency and Overload. Hematology 2003; 1: 40-61.

[7] Beguin Y. Soluble transferrin receptor for the evaluation of erythropoiesis and iron status. Clinica Chimica Acta 2003; 329: 9-22.

[8] Khumalo H, Gomo ZAR, Moyo VM, *et al.* Serum transferrin receptors are decreased in the presence of iron overload. Clinical Chemistry 1998; 44: 40-4.

[9] Jacobsson S. Clinical Value of Serum Transferrin Measurements. The Electronic Journal of the International Federation of Clinical Chemistry (eJIFCC) 2001; 13(2): 1-8.

[10] Rees DC, Williams TN, Maitland K, Clegg JB, Weatherall DJ. Alpha thalassaemia is associated with increased soluble transferrin receptor levels. British Journal of Haematology 1998; 103: 365-9.

[11] Brittenham GM, Weiss G, Brissot P. *et al.* Clinical Consequences of New Insights in the Pathophysiology of Disorders of Iron and Haem Metabolism. Hematology 2000; 39-50.

[12] Pootrakul P, Kitcharoen K, Yansukon P. *et al.* The effect of erythroid hyperplasia on iron balance. Blood 1988; 71: 1124-9.

[13] Heubers HA, Beguin Y, Pootrakul P, Einspahr D, Finch CA. Intact transferrin receptors in human plasma and their relation to erythropoiesis. Blood 1990; 75: 102-7.

[14] Baynes RD, Cook JD, Bothwell TH, Friedman BM, Meyer TE. Serum transferrin receptors in hereditary hemochromatosis and African siderosis. American Journal of Hematology 1994; 45: 288-92.

[15] Thornstensen K. Romslo I. Measurement of serum transferrin receptors in screening for hemochromatosis. Clinical Chemistry 1992; 38: 1510.

[16] Ledue TB, Craig WY. Serum concentrations of transferrin receptor in hereditary hemochromatosis. Clinical Chemistry 1995; 41: 1053-4.

[17] Centis F, Delfini C, Agostinelli F. *et al.* Correlation between soluble transferrin receptor and serum ferritin levels following bone marrow transplantation for thalassemia. European Journal of Haematology 1995; 54: 329-33.

[18] Weiss G. Goodnough LT. Anemia of Chronic Disease. The New England Journal of Medicine 2005; 352: 1011-1023.

[19] Lebron JA, Bennett MJ, Vaughn DE. *et al.* Crystal Structure of the Hemochromatosis Protein HFE and Characterization of Its Interaction with Transferrin. Receptor Cell 1998; 93: 111-123.

[20] Giannetti AM. Bjorkman PJ. HFE and Transferrin Directly Compete for Transferrin Receptor in Solution and at the Cell Surface. The Journal of Biological Chemistry 2004; 279(24): 25866-25875.

[21] Sharma V, Panigrahi I, Dutta P. *et al.* HFE mutation H63D predicts risk of iron overload in thalassemia intermedia irrespective of blood transfusions. Indian Journal of Pathology & Microbiology 2007; 50(1): 82-5.

[22] Viprakasit V, Vathesathokit P, Chinchang W. *et al.* Prevalence of HFE mutations among the Thai population and correlation with iron loading in haemoglobin E disorder. European Journal of Haematology 2004; 73(1): 43-9.

[23] Pietrangelo A. Physiology of iron transport and the hemochromatosis gene. American Journal of Physiology – Gastrointestinal Liver Physiology 2002; 282: G403-G414.

[24] Roy CN. Enns CA. Iron Homeostasis: New tales from the crypt. Blood 2000; 96(13): 4020-4027.

[25] Cazzola M, Beguin Y, Bergamaschi G. *et al.* Soluble transferrin receptor as a potential determinant of iron loading in congenital anaemias due to ineffective erythropoiesis. British Journal of Haematology 1999; 106: 752-5.

[26] Agarwal S, Tewari D, Arya V, Moorchung N, Tripathi R, Chaudhuri G, Pradhan M. Status of HFE mutation in thalassemia syndromes in North India. Annals of Haematology 2007; 86: 483- 485.

[27] Cazzola M, Steffano PD, Ponchio L. *et al.* Relationship between transfusion regimen and suppression of erythropoiesis in β-thalassaemia major. British Journal of Haematology 1995; 89: 473-8.

[28] Olivieri NF. The β-Thalassaemias. The New England Journal of Medicine 1999; 341(2): 99-109.

[29] Rees DC, Porter JB, Clegg JB, Weatherall DJ. Why are Hemoglobin F Levels Increased in HbE/β Thalassaemia? Blood 1999; 9: 3199-204.

[30] Marengo-Rowe AJ. The thalassaemias and related disorders. Proceedings of the Baylor University Medical Centre 2007; 20: 27-31.

[31] Cazzola M, Borgna-Pignatti C, Locatelli F. *et al.* A moderate transfusion regimen may reduce iron loading in beta-thalassaemia major without producing excessive expansion of erythropoiesis. Transfusion 1997; 37: 135-40.

[32] Cook J. The Measurement of Serum Transferrin Receptor. The American Journal of Medical Science 1999; 318(4): 269-79.

[33] Jayaranee S, Sthaneshwar P. Serum soluble transferrin receptor in hypochromic microcytic anaemia. Singapore Medical Journal 2006; 47(2): 138-42.