Is the total number of blood transfusion in β-thalassemia major patients can be used to assess their serum ferritin levels ?

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Abstract

β-thalassemia is an inherited blood disorder in hemoglobin synthesis that results in anemia, growth retardation and certain pathological changes. The treatment of choice for β-thalassemia is the repeated blood transfusion, but unfortunately it results in an iron overload with additional risk factors. The present study aims to evaluate iron profile in transfusion dependent β-thalassemia patients and the effects of repeated blood transfusion on serum ferritin level and the possibility of using the total number of blood transfusion to predict serum ferritin level. 40 transfusion dependent β-thalassemia patients were included in the present study all dependent on blood transfusion only with age range 1-4 years old. A control group of 20 apparently healthy children with age range 1-4 years old was used for comparison. Blood sample of 5 ml was collected from each individual and tested for serum levels of iron, TIBC, % saturation, and serum ferritin. The present study revealed significant changes in the levels of iron profile parameters between patients and control subjects manifested by significantly elevated serum iron and ferritin levels with significantly decreased serum TIBC. Iron status and iron overload in transfusion dependent β-thalassemia patients revealed a significant correlation between serum ferritin level and the number of transfused RBCs units. However this correlation is a rough correlation and some times unpredictable and it could not be used to assess the level of serum ferritin as used by some physicians.

Key words*: *β-thalassemia, blood transfusion, iron profile, ferritin

هل أن العدد الإجمالي لمرّات نقل الدم لمرضى البيتا ثلاسيميا الكبرى يمكن أن يستخدم لتقييم مستويات فريتين مصل الدم لديهم؟

احمد يحيى دلال باشي

الخلاصة

مرض الثلاسيميا نوع بيتا والمعتمد على نقل الدم هو من الأمراض الوراثية، والذي يؤدي إلى اضطراب في تصنيع خضاب الدم مما يؤدي إلى فقر الدم وتأخر النمو وبعض التغيرات المرضية للدم . إن العلاج المثالي هو تكرار نقل الدم والذي يؤدي إلى زيادة نسبة الحديد والذي يزيد من عوامل الخطورة على صحة المرضى . وهذه الدراسة تهدف إلى تقييم هيئة الحديد وتأثيرات نقل الدم المتكرر لدى مرضى الثلاسيميا الكبرى نوع بيتا على مستوى فرتين مصل الدم ، وإمكانية تقييم كمية فرتين مصل الدم بواسطة احتساب عدد نقلات الدم للمريض . شملت هذه الدراسة 40 مريضا بالثلاسيميا الكبرى نوع بيتا تتراوح أعمارهم من 1-4 سنوات، وجميعهم

معتمدون على نقل الدم الدوري لهم و 20 مشارك من الأطفال الأصحاء وبأعمار تتراوح بين 1-4 سنوات كمجموعة ضابطه. تم اخذ نموذج من الدم مقداره 5 مل من جميع المشاركين، وتم فصل مصل الدم. وأجريت على كل نموذج لمصل الدم فحوصات مستوى الحديد، نسبة التشبع بالحديد ، القدرة الكلية لارتباط الحديد، وكمية الفريتين. أثبتت هذه الدراسة وجود تغيرات معنوية في المعلمات لهيئة الحديد بشكل عام بين مرضى الثلاسيميا الكبرى نوع بيتا ممن يعتمدون على نقل الدم فقط وبين المجموعة الضابطة، حيث كانت الزيادة معنوية وواضحة في حديد مصل الدم وفريتين مصل الدم مع نقص معنوي في القدرة الكلية لارتباط الحديد في مصل الدم . إن وضعية هيئة الحديد وزيادة الحديد في مرضى الثلاسيميا الكبرى نوع بيتا ممن يعتمدون على لنقل الدم الدوري لهم أوضحت وجود علاقة ارتباط معنوية بين مستوى فرتين مصل الدم وعدد نقلات الدم . ورغم ذلك فلا يمكن الاعتماد على عدد النقلات في تقدير كمية فرتين مصل الدم حيث يكون التقدير تقريبي وأحيانا غير متوقع ولا يمكن الاعتماد عليه في احتساب كمية فرتين مصل الدم كما يفعل بعض الأطباء.

Introduction

β-thalassemia, one of the most widespread genetic disease in the world, is an autosomal hematological disorder that is the result of genetically absent or deficient synthesis of the beta-globin chains of hemoglobin [1, 2]. β-thalassemia affects many population groups all over the world with about 5% carrier rate [3]. It is most prevalent around the Mediterranean sea countries, also the Middle East, the Indian subcontinent and other countries[4]. β-thalassemia is caused by more than 200 different point mutations and are extremely heterogeneous[5, 6]. According to the mutation affecting the 2 beta-globin genes and the severity of symptoms, β-thalassemia is classified into β-thalassemia major, minor, and intermedia [7]. β-thalassemia major, or transfusion dependent β-thalassemia, is the most sever form of β-thalassemia, characterized by ineffective erythropoiesis, grossly defective synthesis of hemoglobin A (HbA), impaired red blood cell (RBC) production, increased hemolysis of the defective RBCs, and anemia [8, 9]. The treatment of choice for β-thalassemia major patients is a life long regular blood transfusion regimen [10]. It consists of 1-3 units of RBCs every 2-4 weeks since early childhood [11]. This treatment option is effective in correcting anemia and other pathological features associated with the disease, and should be aimed to keep a minimum hemoglobin level above 10 g/dl, thus improving survival of the patients [12, 11]. However, lifesaving repeated RBC transfusion may carry certain complications to β-thalassemia patients, the most relevant of these is iron overload [13]. Chronic transfusion therapy may delivers 0.4-0.5 mg/kg/day of iron, and the patients will become iron overloaded within relatively short period of therapy, whereby iron chelation therapy is needed to prevent deleterious consequences of iron overload [14]. Deferoxamine has been the standard iron chelator, used for prolonging and improving the quality of life of transfusion dependent β-thalassemia patients [15]. When the serum ferritin level reaches at 1000 ng/ml, it is generally taken as the point to initiate iron chelation therapy. The iron burden on the body can be estimated by means of serum ferritin, iron and TIBC levels. The estimation of serum ferritin levels is the most commonly employed test to evaluate iron overload in β-thalassemia major [16]. The present study aims to evaluate the level of serum ferritin and iron profile in transfusion dependent β-thalassemia major patients, and the effects of multiple blood transfusion on serum ferritin level and the possibility of using the total number of blood transfusion to predict serum ferritin level.

Patient and methods

- ***Patients (group A):***

A total of 40 patients of age range 1-4 years old, all are transfusion dependent β-thalassemia patients attending the Thalassemia Center in Ibn-Alatheer Teaching Hospital were enrolled in this study, since the 4th of December 2010 to the 1st of June 2011. The patients were diagnosed as having β-thalassemia major depending on Hb variant test using high performance liquid chromatography (HPLC).

- ***Control (group B):*** consist of 20 non-thalassemic individuals all are apparently healthy with age range 1-4 years old for comparison.
- ***Specimens and methods***:

Venous blood sample (about 5 ml) was collected from each individual of the studied groups in a plain tube. The tubes are placed in a water bath at 37°C for 15 minutes for blood clotting to occur. Serum samples were obtained by centrifugation of blood samples at 4000 rpm for 10 minutes. The serum was divided and placed in 1 ml eppendroff tubes then freezed at -20°C. All the biochemical analysis were performed at the laboratory of higher studies in the Department of Biochemistry, College of Medicine, University of Mosul, Mosul, Iraq. The selection of reagents used in this study was based on accuracy, reliability, availability, and were purchased as kits.

Serum iron was measured by Jaffe reaction method [17], using a kit supplied by Randox laboratories (UK), by means of UV-VIS spectrophotometer (PD-303 UV, Japan).

Serum TIBC was measured by enzymatic method [18], using a kit supplied by Biomerieux (France).

Serum ferritin was measured by an enzyme linked assay method [19] using a kit supplied by Biomerieux (France), and to be tested automatically with Minividas, Biomerieux (France).

- ***Statistical analysis***:

The standard statistical methods for the analysis of data in this study were used to determine the mean, standard deviation (SD), unpaired t-test, in addition to non linear regression [20]. The statistical results were considered significant at $P \leq 0.05$ [21].

Results

The results of the present study revealed significantly higher mean serum levels of iron and ferritin ($P \leq 0.01$), with high significantly decreased serum TIBC level ($P \leq 0.01$) in group A β-thalassemic patients when compared with control subjects in group B (table 1). The comparison between subgroups of different age ranges in group A showed significantly increased mean serum ferritin level ($P \leq 0.05$). The mean value of transferrin saturation % was not significantly increased. In addition, no significant changes observed in the mean serum levels of iron and TIBC. The number of blood transfusions was significantly increased in older subgroup of patients ($P \leq 0.01$) (table 2). The relation between serum ferritin level and the number of transfused RBCs units was expressed by the non-linear or cubic regression as shown by figure (1) and represented by the regression equation:

$$y=a+b_1X+b_2X^2+b3\ X^3$$

The equation revealed the effects of blood transfusion on serum ferritin level where:

y= serum ferritin

a= constant (represent the level of serum ferritin before any blood transfusion)

X= number of transfused RBC units

b_1= constant (represent the amount of serum ferritin elevated per each transfused RBC unit.

b_2 and b_3= constants

According to the data obtained, the regression equation was set to be as follows:

Estimated serum ferritin= $83.2+ 316.2X + 11.8X^2 +(– 0.164)X^3$

So that, according to the regression equation, each transfused packed RBC unit could elevate the serum ferritin level by about 316.2 ng/ml. and by using this equation, serum ferritin is supposed to be calculated and estimated depending on the number of transfused RBC units. However, the results revealed a wide variety between the measured and calculated serum ferritin in those patients after application of this equation.

Table (1):- Differences between group A and group B regarding iron profile.

Serum Parameters	Mean ± SD		P- Value
	Group A	Group B	
Iron	25.17 ± 10.3	7.5 ± 4.9	0.000*
TIBC	35.12 ± 14.9	50.6 ± 7	0.006*
Ferritin	2669.4 ± 1667.7	60.19 ± 53.8	0.000*

Table (2):- Differences between age subgroups of β-thalassemia patients in group A.

Serum Parameters	Mean ± SD		P- Value
	≤ 2 years	> 2 years	
Iron	25.15 ± 11.15	25.19 ± 9.32	NS
TIBC	34.6 ± 14.1	35.8 ± 16.5	NS
Ferritin	2149.26± 1199.5	3123.4 ± 1411.9	0.023*
Saturation %	67 ± 41.8	94.5 ± 68.4	NS
No. of transfusions	12.5 ± 6	22.18 ± 11.7	0.001*

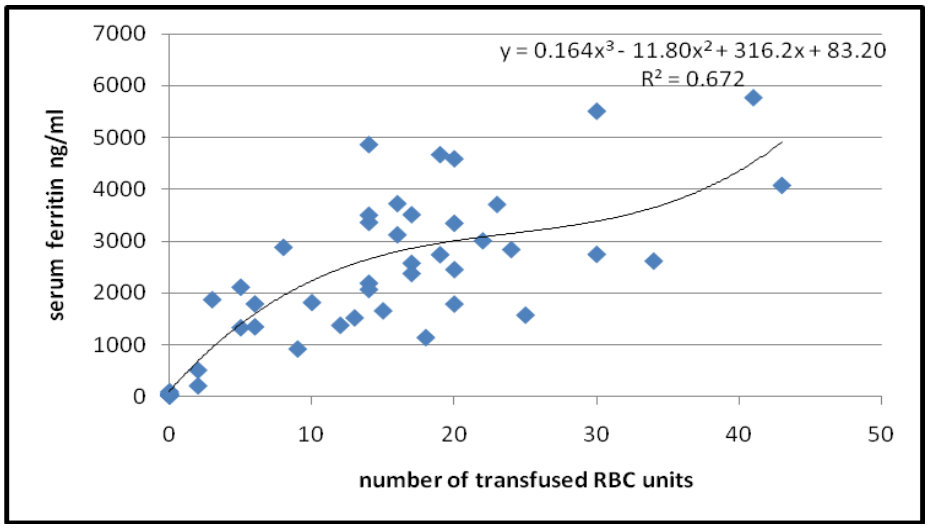

Figure (1):- Regression between serum ferritin level and number of blood transfusions in group A.

Discussion

It is obvious that significant differences existed between transfusion dependent β-thalassemia patients and the control subjects concerning iron profile. These significant differences are definitely caused by repeated blood transfusion program since early childhood in these patients. So that, a significant increase in the mean serum iron and ferritin ($P \leq 0.01$) is observed which is related to the large amounts of iron entering the body per each unit of transfused RBCs, and that is augmented by increased iron absorption [22]. The result is a saturation of transferrin in the blood by overloaded iron leading to increased level of non-transferrin bound iron (NTBI) which will eventually precipitated in the tissues [23]. In transfusion dependent β-thalassemia patients, the significant increase in the mean serum ferritin level was combined with a significant increase in the mean number of transfused RBCs units when the results were compared between different age subgroups of patients. These results refer to the tight correlation existed between serum ferritin level and number of transfused RBC units. It also prove the validity of measuring serum ferritin as an indicator of iron overload in transfusion dependent β-thalassemia patients. These results are in accordance with that of Telfer *et. al.*, [24], as their results prove the effects of blood transfusion on increasing serum ferritin level and the validity of measuring serum ferritin to indicate iron status in the body. The regression equation between the number of transfused RBCs units and serum ferritin, by expressing the effects of a single blood transfusion on serum ferritin level may give a rough estimation about serum ferritin level in β-thalassemia patients depending on the number of transfused RBCs units before starting an iron chelating therapy. However it is not advised to depend on such estimation as it can give unpredictable results and it could not be used to assess the level of serum ferritin as used by some physicians. Although blood transfusion to the patients constitute the major factor in elevating serum ferritin level, however, other factors may also affect the level of serum ferritin to a certain degree e.g. intercurrent infection and inflammation. This situation make it inaccurate to estimate the level of serum ferritin depending on the number of transfused RBCs units [11, 25].

Conclusion

Iron profile measurement shows a significant alteration in transfusion dependent β-thalassemia patients compared with control subjects. In addition, a significant correlation was existed between serum ferritin and the number of transfused RBC units in transfusion dependent β-thalassemia patients. However, it is difficult to estimate serum ferritin level depending only on the number of transfused RBC units.

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