ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Assesment of Transferrin Saturation as an Indicator of Iron Overload in Homozygous & Hetrozygous Form of Thalassemia.

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ABSTRACT

β thalassemia syndromes are a group of hereditary disorder characterized by genetic deficiency in the synthesis of β- globin chains. It is associated with increase in iron overload which becomes one of the major causes of morbidity & mortality. When serum iron and serum TIBC are used to diagnose iron overload, the measurements are usually combined in the calculation of serum transferrin saturation. We measured serum iron, total iron binding capacity & calculated serum transferrin saturation, unbound iron capacity level in thalassemia major & minor to assess the iron status among groups. 25 cases of β thalassemia major & minor diagnosed by hemoglobin electrophoresis and 25 age matched healthy subject were included in the study. serum iron levels were significantly ($p< 0.05$) elevated in thalassemia major but remain insignificant for thalassemia minor when compared with healthy subjects. Serum TIBC increased in major & remains insignificant in minor form of thalassemia when compared to control. Transferrin saturation increased in both homozygous & heterozygous form of thalassemia significantly as compared to control. Transferrin saturation may be the best way to utilize the information about iron overload & development of complications like oxidative stress due to non-bound iron form.

Keywords: β-Thalassemia– serum iron - Transferrin saturation –Iron overload

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ISSN: 0975-8585

INTRODUCTION

Thalassemia is a genetic disease associated with hemoglobin, defect in β-globin chain synthesis. The beta thalassemia carrier rate in India is around 3-7% with higher frequency in certain ethnic groups. [1] Thalassemia major is the severe transfusion dependent form and major cause of morbidity & mortality in these thalassemic are anemia & iron overload. [2] The progressive iron overload observed in β thalassemia major patients is the side effect of ineffective erythropoiesis, increased gastrointestinal absorption of iron, lack of physiological mechanism for excreting excess iron & multiple blood transfusions, which results in Hemochromatosis. Even elevated body iron load is observed in milder form of thalassemia. [3] Although the survival of thalassemic is steadily increasing, the prevalence of complications due to iron over load remains high. Thus Iron overload complication in β thalassemia is the major focus of management today. [4]Repeated laboratory assessment of iron status is necessary for monitoring iron overload in thalassemia.

Routinely Serum Iron concentration and Total iron binding capacity (TIBC) reflects the iron status of the body, however several additional factors influence their value in serum. TIBC indirectly measures transferrin a specific carrier protein, which increase as serum iron concentration (and stored iron) decreases. Unfortunately, result of this parameter is also affected by factors like malnutrition, inflammation, chronic infection, and cancer. Ferritin is a storage compound for iron, and serum ferritin levels normally correlate well with total iron stores. However, due to limited availability and high cost factor and being an acute-phase reactant which can be elevated in the setting of inflammation, chronic infection, or other diseases, measurement of ferritin levels remain limited. [5] Thus single test may not be able to evaluate the correct status of actual iron overload of an individual as many factors not concern influence on them. Transferrin saturation (Tf sat) is a calculated parameter obtained from two measured values i.e. serum iron concentration divided by TIBC, and expressed as a percent. Thus by combining these measurements i. e. percent transferrin saturation can be used to assess the iron overload & level of serum Ferritin i.e. iron store of the body effectively. [6]

Percent transferrin saturation may be useful tool in clinical practice if properly evaluated. It would be ideal because it will not be influenced by other factors or their effect will be minimal. Present study was undertaken to evaluate percent transferrin saturation in both homozygous & heterozygous form of thalassemia to estimate the toxic effect of iron or iron overload in non transfused patients. This calculated parameter may provide guide for Iron chelation therapy and help to minimize further complications caused by excess iron.

MATERIALS AND METHODS

Clinically diagnosed cases of β thalassemia major (n= 25) & β thalassemia minor (n=25) having severe anemia were included in study group. Diagnosis of β thalassemia was made by clinical examination of hepatospleenomegaly, RBC indices. Diagnosis was confirmed by Hb F and HbA_2 concentrations on hemoglobin electrophoresis. Age matched healthy subjects were selected for control group. Study population was divided into following three groups,

Group A: Subjects diagnosed with β thalassemia major (25) and having Hb F 30-50 %, HbA_2 5-10%

Group B: Subjects diagnosed with β thalassemia Minor (25) and showing Hb F1-3 %, HbA_2 2-7%, Hb A -90-95%

Group C: Normal healthy subjects having Hb level > 11 gm %. Hb A 95-97% and Hb A_2 2.5 - 3.0% having no HbF band on electrophoresis also did not show any bleeding disorder.

Individuals with sickle cell anemia, Iron Deficiency Anemia or bleeding disorders were excluded from the study

Collection of Blood Sample: 7 ml of blood sample was drawn, out of which 2ml blood was collected in EDTA bulb for RBC indices and Hemoglobin electrophoresis. Peripheral smear was prepared on the fresh blood. Precaution was taken to avoid any traces of hemolysis. Remaining 5ml of blood was transferred to plain tube and allowed for clotting. After half an hour clear serum was separated by centrifugation at 3000 rpm for 5 min. Serum samples were used for iron &Total iron binding capacity (TIBC) tests.

Hemoglobin electrophoresis was performed on cellulose acetate paper at alkaline pH 8.6 using tris EDTA borate buffer, HbF estimation was carried out by alkali denaturation method (modified betke method) and by scanning hemoglobin electrophoresis gel. Serum iron & Serum TIBC were estimated by Giovanniello& peters method of Bathophenanthroline [7]

Percent Transferrin saturation was calculated by the formula, Percent Transferrin saturation = (Serum iron/ Serum TIBC) x 100. [8]

Student's t test was employed for statistical analysis. Comparison of the data between study & control group were done and were expressed as mean ± SD. Pearson's correlation coefficients were used to observe correlation between two parameters.

RESULTS

Red cell indices and Biochemical parameters of control & study group are given in table no. 1. Highly significant difference was observed in hemoglobin levels between control and both β thalassemia groups. Significant increase in serum iron was observed in β thalassemia major group (26.16±6.56 µmol/L) whereas mild increase of serum iron remained non-significant in β thalassemia minor group (19.95±8.12 µmol/L) when compared with control (18.26±3.77µmol/L).Observed rise of serum TIBC in β thalassemia major(57.06±5.06 µmol/L) compared to control group (50.73±7.01 µmol/L) remain statistically significant. Calculated parameter, percentage transferrin saturation when compared with control was observed significantly elevated in both thalassemia major & minor group (33.86±7.20 vs 49.45±14.67 & 43.08±12.09). There was no significant difference observed in calculated parameters Serum UIBC in both β thalassemia groups when compared with control.

ISSN: 0975-8585

Table No.1 – Red blood Cell indices & Biochemical parameters of control & study group

Demographic Criteria	Control group (n= 25)	Thalassemia Major (n= 25)	TThalassemia Minor (n= 25)
Hbgm %	11.89±0.82	5.48±1.86 **	6.39±2.42**
Hb F	0.81±0.64	34.62±17.63	6.82±8.23
Hb A2	2.38±1.12	4.32±1.68	4.28±1.20
MCVfl	85.3±6.8	70.18±6.65	66.38±9.89
MCHpg	25.3±3.6	23.92±5.58	20.78±5.41
MCHC %	31.1±3.8	33.28±6.48	28.8±4.27
Serum iron µmol/L	18.26±3.77	26.16±6.56*	19.95±8.12NS
Serum TIBC µmol/L	50.73±7.01	57.06±5.06*	51.17±17.12NS
Serum UIBC	33.45±7.27	30.84±16.43NS	30.58±18.24NS
Transferrin saturation %	33.86±7.20	49.45±14.67*	43.08±12.09*

a) * $p < 0.05$ - significant compared to control
b) ** $p < 0.001$- highly significant compared to control
c) NS – non significant

Statistically significant positive correlation was observed between percentage transferrin saturation & serum iron in thalassemia major.

DISCUSSION AND CONCUSION

Transferrin saturation value (normal 20-35%) is more consistently helpful than either value of serum iron or serum TIBC alone. In both children and adults value < 5% is diagnostic of iron deficiency, while <16% is suggestive of iron deficiency or for anemia of chronic disorders. However it does not reflect iron stores, & it is related to efficiency of moving iron out of iron processing cells like reticuloendothelial macrophages, hepatocytes or absorptive erythrocytes. [9]

Most times, serum ferritin levels are related to the quantity of iron stored in the body with and without iron overload. Serum ferritin is a useful screening test for the initial diagnosis of thalassemia. [10] However, serum Ferritin protein is an acute phase reactant, rising with any inflammation process from infection through chronic disease like acute or chronic inflammatory processes, autoimmune diseases, neoplasias, chronic renal insufficiency, hepatopathies, and metabolic syndrome. So increase in ferritin concentrations with no excess iron body level can be observed in terms to determine whether a high serum ferritin protein is due to iron overload or inflammation; in these conditions transferrin saturation generally is normal or decreased. On the other hand, when there is an iron overload, the increase in ferritin concentrations is associated with increased saturation of transferrin. [11] It has been also necessary to determine

transferrin saturation, as Transferrin has a much longer half life in plasma than iron and shows short term of fluctuation .Transferrin can be measured indirectly as the ability of the plasma protein to bind iron so called TIBC. [12]

The progressive iron overload in β thalassemia major patients is the consequence of ineffective erthyropoiesis, increased gastrointestinal absorption of iron, lack of physiologic mechanism for excreting excess iron, and above all multiple blood transfusions. The iron which exceeds the iron binding capacity of transferrin appears in the plasma as non-transferrin bound iron, which is highly toxic to tissues [13]. The accumulation of iron results in progressive dysfunction of the heart, liver and endocrine glands. The iron burden on the body can be assessed by means of elevated levels of serum transferrin saturation, serum ferritin, iron and TIBC levels. As loading continues, the capacity of transferrin, the main transport protein of iron, to bind and detoxify this essential metal may be exceeded. The resulting nontransferrin-bound iron (NTBI) fraction within plasma may promote the generation of reactive oxygen species (ROS), propagators of oxygen-related damage.[14,15] Iron overload is responsible for the most damaging effects of the thalassemias, making iron chelation a focal point of the management of this diseases.

We calculated S-Unbound Iron Binding Capacity (S-UIBC) as s-TIBC minus s-iron, after measuring s-transferrin saturation and calculating s-TIBC.S-UIBC may also be directly analyzed. Our results showed s-UIBC fails to explain diagnostic accuracy for free iron non bound iron in plasma as that of transferrin saturation. Our results correlated to the Arne et al [16]

In nontransfused patients with severe thalassemia, abnormal dietary iron absorption results in an increased body iron burden between 2 and 5 g per year depending on the severity of erythroid expansion. If regular transfusions are required, as in β-thalassemia major patients, this doubles the rate of iron accumulation. [17] In the severe forms of beta-thalassemia, multiple blood transfusions and a deficiency of a potent iron absorption inhibitor, result in iron overload with increased ferritin serum concentration and transferrin saturation. [18] In the milder forms, ineffective erythropoiesis is discreet and these individuals rarely present iron excess. [19]

Differentiating between the iron overload & severe anemia in thalassemias in children are important. Not only does an appropriate diagnosis; allow adequate management, appropriate family counseling & have important prognostic implication, but it also prevents unnecessary iron therapy at increased risk of iron toxicity.

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