
ORIGINAL ARTICLE**Assessment of Iron Overload in Homozygous and Heterozygous Beta Thalassemic Children below 5 Years of Age***Dhiraj J. Trivedi^{1*}, Aparna Sagare²**¹Department of Biochemistry, SDM College of Medical Sciences and Hospital, Sattur, Dharwad - 583009 (Karnataka) India, ²Department of Biochemistry, Smt. Kashibai Navale Medical College and General Hospital, Pune - 411041 (Maharashtra) India.***Abstract:**

Background: Thalassemia is a genetic disease having 3-7% carrier rate in Indians. It is transfusion dependent anemia having high risk of iron overloading. A clinical symptom of iron overload becomes detectable in second decade causing progressive liver, heart and endocrine glands damage. There is a need to assess iron overload in thalassemics below 5 years of age to protect them from complications at later age of life. *Aims and objectives:* Present study was undertaken to estimate serum iron status and evaluate serum transferrin saturation in both homozygous & heterozygous form of thalassemia as an index of iron overload among children of one to five years of age. *Materials and Methods:* Clinically diagnosed thirty cases of β thalassemia major & thirty cases of β thalassemia minor having severe anemia, hepatosplenomegaly and between 1 year to 5 years of age were included in study group and same age matched healthy controls were included in the study. RBC indices and HbA, HbA2 and HbF were estimated along with serum iron & serum Total Iron Binding Capacity (TIBC) and serum transferrin levels. *Results:* Significant difference was observed in hemoglobin levels between control and both beta thalassemia groups. Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) values were reduced. Hemoglobin electrophoresis showed the elevated levels of HbF and HbA2 in both beta thalassemia groups. Among serum iron parameters, serum iron, TIBC and transferrin saturation were elevated whereas serum transferrin levels were low in thalassemia major in children below 5 years of age. *Conclusion:* Although clinical symptoms of iron overload have been absent in

thalassemic children below five years of age, biochemical iron overloading has started at much lower age which is of great concern.

Keywords: Hemoglobinopathy, Iron overload, Thalassemia.

Introduction:

Thalassemia is a genetic disease associated with disproportionate globin chain synthesis. Based on affected globin chain it may be alpha thalassemia or beta thalassemia. Beta thalassemia carrier rate in India is around 3-7%, though higher frequency is seen in certain ethnic groups [1]. Thalassemia major is the severe transfusion dependent form of anemia. Major causes of morbidity & mortality in thalassemics are anemia and iron overload [2]. The progressive iron overload in thalassemics is the side effect of ineffective erythropoiesis, increased gastrointestinal absorption of iron, lack of physiological mechanism for excreting excess iron and multiple blood transfusions resulting in hemochromatosis. Even elevated body iron overload is observed in milder form of thalassemia [3]. Though survival of thalassemics is steadily increasing, the prevalence of complications due to iron overload remain high. Typically, the symptoms of iron overload become detectable in second decade of life but, non-transferrin bound fraction of iron may have started accumulating from early life and its toxic effects progressively damage heart, liver and endocrine glands at later age [4].

Thus management of iron overload complication is the major focus of thalassemia treatment today [5].

Repeated laboratory assessment of iron status is necessary for monitoring and preventing of iron overload in thalassemia. Routinely serum iron concentration, Total Iron Binding Capacity (TIBC) and serum ferritin reflects the iron status of the body [6]; however several additional factors influence their value in serum. TIBC level increases in iron deficiency anemia whereas it is decreased in anemia of chronic diseases. Unfortunately, results of TIBC are also affected by factors like malnutrition, inflammation or chronic infection, which are common in thalassemics. Ferritin is a storage compound for iron and serum ferritin levels normally correlate well with total iron stores, however, restricted availability and cost factor brings limitations in estimation. Additionally, serum ferritin is an acute-phase reactant, gets elevated in inflammation, chronic infection, or even in iron deficiency anemia.

TIBC indirectly measures serum transferrin, a specific iron carrier protein. Serum transferrin saturation (Tfsat) is a calculated parameter obtained from serum iron concentration divided by TIBC and expressed as a percent. Serum transferrin saturation is commonly used indicator for inclining iron over load. Serum transferrin saturation reflects true status of body iron as values are not influenced by other factors or their effect will be minimal. Serum transferrin saturation correlates well with the level of serum ferritin and hence the iron store of the body [7].

Present study was undertaken to estimate serum iron status and evaluate serum transferrin saturation in both homozygous & heterozygous form of thalassemia as an index of iron overload among children of one to five years of age. The study also included correlation of serum iron and serum transferrin saturation, a calculated parameter, to

have insight of iron overload status. This may assist as a guide to minimize the toxic effect of iron overload developing at later age in thalassemics.

Material & Methods:

Present study of assessment for iron overload in beta thalassemics was conducted by Department of Biochemistry of SDM College of Medical Sciences and Hospital, Dharwad, Karnataka, India. Clinically diagnosed thirty cases of β thalassemia major & thirty cases of β thalassemia minor having severe anemia, Hepatosplenomegaly between 1 to 5 years of age were included in study group. Same age matched healthy children were selected as control group. The informed written consent concern forms were collected from parents of patients & normal group children. The present study was approved by Institutional Ethics Committee. Diagnosis of β thalassemia was made first by clinical examination of hepatosplenomegaly, RBC indices and confirmed by estimating HbF and HbA₂ concentrations on hemoglobin electrophoresis. Study population consisted of following three groups,

Group A: Subjects diagnosed as β thalassemia major (n=30) due to HbF more than 30%, HbA₂ more than 7% and age between 1 to 5 years.

Group B: Subjects diagnosed as β thalassemia minor (n=30) based on levels of HbF 1-3%, HbA₂ 2-7%, HbA -90-95% and age between 1 to 5 years.

Group C: Control healthy children, age group 1 to 5 years having HbF less than 1% and HbA₂ level less than 3.5%. Children who did not show any bleeding disorder or anemia were included as control. Patients with sickle cell anemia, iron deficiency anemia, jaundice and any bleeding disorders were excluded from the study.

Collection and Analysis of Blood Samples:

Out of 5 ml blood sample collected by taking aseptic precaution, 2 ml was transferred to EDTA

tube and remaining 3 ml was transferred to plain tube for serum separation. Precaution was taken to avoid any traces of hemolysis. A direct drop of blood was used for peripheral smear.

2 ml blood collected in EDTA was used for RBC indices (Sysmax Transasia Co.) and hemoglobin electrophoresis. Hb electrophoresis was performed at alkaline pH 8.6 using TBA buffer. HbA, HbA2 and HbF were estimated by Platinum software provided by Helena – Alere diagnostics US. HbF was also estimated by alkali denaturation method modified by Betke [8].

3 ml of blood collected in plain tube was allowed to clot. After an hour clear serum was separated by centrifugation at 3000 rpm for 10 min & serum samples were analyzed for serum iron & serum TIBC by Giovanniello & Peters method of Bathophenanthroline [9]. Serum transferrin saturation was calculated by = (Serum iron / Serum TIBC) x 100 [10]. Serum transferrin levels were estimated by Calorimetric method suggested by Lung Nan Lin *et al* [11].

Statistical Analysis:

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

Results:

Table 1 shows blood indices and hemoglobin levels in study groups.

Highly significant difference ($P < 0.001$) was observed in hemoglobin levels between control and both beta thalassemia groups. Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) values were reduced. On electrophoresis, significantly elevated ($p < 0.001$) levels of HbF and HbA2 were observed in beta thalassemia major group, whereas in case of beta thalassemia minor levels of HbF and HbA2 showed statistically significant ($p < 0.05$) mild increase.

Among 90 children included in the present study, 30 children were age matched controls and 30

Table 1: Blood Indices and Hemoglobin Levels in Control and Study Groups

Demographic Criteria	Control Group (n= 30)	Thalassemia Major (n= 30)	Thalassemia Minor (n= 30)
Age (Years)	1 – 5	1 – 5	1 – 5
Hb (gm %)	11.89 \pm 0.89	5.44 \pm 2.38**	7.36 \pm 2.88**
HbF	0.82 \pm 0.74	34.8 \pm 18.64**	4.29 \pm 1.53*
HbA2	2.38 \pm 1.12	6.71 \pm 8.62*	4.3 \pm 1.01*
MCV (fl)	86 \pm 6.2	69.18 \pm 7.09	66.36 \pm 10.39
MCH (pg)	25 \pm 3.6	23.97 \pm 5.58	20.67 \pm 5.80
MCHC (%)	30 \pm 4.4	32.27 \pm 6.44	29.8 \pm 4.03
H/O Number of units of blood transfusion	Nil	13.52 \pm 3.43 (Min-04; Max-19)	2.84 \pm 1.25 (Min-01; Max-04)

* $p < 0.05$ - significant compared to control, ** $p < 0.001$ - highly significant compared to control, NS – non significant

children each belonging to thalassemia major and thalassemia minor groups. Age of the patients at the time of this study ranged in between 1 to 5 years (Mean age 2.38 years).

Table 1 also shows that thalassemia major had higher degree of anemia as compared to thalassemia minor group. This anemia was treated by blood transfusion. When history of blood transfusion among thalassemia major and minor was compared thalassemia major had received higher number of units of blood (13.52 ± 3.43) as compared to thalassemia minor group (2.84 ± 1.25).

Table 2 describes serum iron parameters in control and study groups. When compared with controls, significant ($p < 0.05$) increase in serum iron was observed in β thalassemia major group whereas

beta thalassemia major group illustrated significant decrease ($p < 0.001$) as compared with serum value of control subjects however, thalassemia minor presented mild decrease without any statistical significance.

Discussion:

Thalassemia is a genetic disease where individuals show iron overload with anemia. This is due to impropportionate globin chain synthesis leading to decreased normal hemoglobin levels. Microcytic anemia is a common observation in thalassemia. Beta thalassemia major which is due to homozygous deletion of beta globin chain gene and patient suffers severe hemolytic anemia, whereas beta thalassemia minor is heterozygous form, which remains asymptomatic or with mild form of

Table 2: Serum Iron Parameters in Control and Study Groups

Demographic Criteria	Control Group (n= 30)	Thalassemia Major (n= 30)	Thalassemia Minor (n= 30)
Serum Iron ($\mu\text{mol/L}$)	21.01 ± 4.87	$24.58 \pm 7.611^*$	$17.95 \pm 8.34^{\text{NS}}$
Serum TIBC ($\mu\text{mol/L}$)	50.38 ± 7.067	$57.61 \pm 18.78^*$	$51.64 \pm 17.62^{\text{NS}}$
Serum Iron/TIBC ratio	0.42 ± 0.11	$0.45 \pm 0.18^{\text{NS}}$	$0.37 \pm 0.19^{\text{NS}}$
Transferrin Saturation (%)	39.06 ± 8.9	$46.78 \pm 16.56^*$	$37.19 \pm 21.57^{\text{NS}}$
Serum Transferrin (mg/dl)	220.6 ± 13.87	$180.7 \pm 36.67^{**}$	$208.2 \pm 43.4^{\text{NS}}$

* $p < 0.05$ - significant compared to control, ** $p < 0.001$ - highly significant compared to control, NS – non significant

observed decrease in serum iron was non-significant in β thalassemia minor group. Upsurge in serum TIBC in β thalassemia major compared to control group was statistically significant ($p < 0.05$) whereas once again beta thalassemia minor did not show any significant change.

A calculated parameter, serum transferrin saturation values were significantly increased ($p < 0.05$) in thalassemia major group, but decreased in thalassemia minor but remained non-significant compared to control. Serum transferrin levels of

anemia. Beta thalassemia minor usually show microcytosis with normal or increased RBC count. Elevated HbA2 is found in iron deficiency but, elevated HbA2 with normal to mildly elevated HbF level is diagnostic and point towards thalassemia, which coincides with our findings. Scientific reports state that decreased level of MCV and MCH [12,13,14] with high HbA2 is indicative of beta thalassemia major while, higher than normal amount of both HbA2 and normal HbF means a milder form of thalassemia. Very low

HbA and high level of HbF points to severity of thalassemia. Thus elevated level of HbA2 is the reliable marker of heterozygous beta thalassemia [15]. Our results correlate with existing scientific reports that beta thalassemia minor exhibits milder form of anemia sometimes it goes asymptomatic throughout whereas, thalassemia major is severe transfusion dependent anemia. Increased serum iron levels are observed in beta thalassemia major but not in minor, may be because of unrecognizably deranged erythropoiesis in milder form of thalassemia where subjects rarely present with iron excess [16] whereas in addition to deranged erythropoiesis, thalassemia major needs supplement of blood which loads extra iron. Transferrin saturation is the amount of transferrin bound to iron. In case of normal individual transferrin saturation is in the range of 20% to 50% but serum transferrin saturation more than 50% is suggestive of iron over load. Serum transferrin saturation in children with beta thalassemia major remains at higher side. In present study, it may be due to severe forms of beta thalassemia which is associated with multiple blood transfusions and deficiency of hepcidin resulting in iron overload and the same is reflected in the form of increased serum transferrin saturation. If these levels reach above 85%, it is referred to as labile plasma iron. This is redox active and responsible for iron related toxicity. This may be the cause of clinical symptoms appearing after gradual overload during the course of two decades. Our results correlate well with the findings of Edwards CQ, *et al* [17] and Fargion *et al* [18] A significant positive correlation has been observed between transferrin saturation & serum iron in thalassemia major. Transferrin, a glycoprotein, controls the level of free iron in biological fluid [19]. Increased levels are indicative of iron deficiency anemia while decreased value points to iron overload diseases. Results of present study once again point at inclined overloading of iron in thalassemic

children at much early age. Children with beta thalassemia need repeated blood transfusions to maintain their hemoglobin level. Transfused blood loads iron in their body, which remains as non-transferrin bound iron and propagates oxygen related damage [20] unless made to excrete. Though clinical symptoms of iron overload appear in second decade of life [21] in present study children below five years of age have not shown any severe clinical symptoms as they may be in the initial phase of iron over loading. But the biochemical iron overloading is apparent from the mildly decreased value of serum transferrin in thalassemia minor while, more obvious in thalassemia major due to observed moderately decreased transferrin levels. Surviving beta thalasseemics may suffer from such iron overload complications [22] because of poorly managed transfusion. Treatment of beta thalassemia needs proper management of iron overload to prevent complication and early mortality.

Conclusion:

Although clinical symptoms of iron overload are absent in thalassemic children below five years of age, biochemical iron overloading has started at much lower age which is of great concern. Differentiating between the iron overload & severe anemia in thalassemic children are important not only for prognostic implication but also to avoid risk of iron toxicity. Prevalence of complications due to iron overload observed in thalasseemics is the life limiting complication which needs to be corrected for the longevity of suffering children.

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