Original article:
Estimation of serum ferritin and TSH levels in thalassemia patients undergoing iron chelation therapy.
Soma Ghosh¹, Dwaipayan Chakrabarti²

Abstract:
Objectives & introduction: Thalassemia, heterogenous group of disorders of haemoglobin, characterised by reduced or absent production of one or more of globin chains. Regular red cell transfusion with chelation therapy for iron overload are cornerstones of therapy for β thalassemia major. Serum ferritin assay is widely available, relatively inexpensive method for assessing body iron burden and monitoring response to chelation process which in turn also improves TSH levels in thalassemic subjects. The objective of this study was to assess prechelation and postchelation levels of serum ferritin and TSH and correlating post chelation levels of serum ferritin and TSH in thalassemic patients >6yrs undergoing chelation therapy.

Materials & methods: Serum TSH measured by Enzyme linked fluorescent assay and serum ferritin measured by enzyme linked immunosorbent assay. Results: Amongst 500 participants, 47% were males & 53% females. Mean age was 9.04 yrs; prechelation ferritin and TSH levels were 2995.78ng/ml with SD of 802.53 and 5.07 µU/ml with SD of 2.52. The postchelation ferritin and TSH levels were 2168.80 ng/ml with SD of 1335.89 and 4.51µU/ml with SD of 4.76. Paired t test with respect to pre and postchelation ferritin and TSH levels showed 2 tailed p as 0.000 and t>3, both of which considered significant. While correlating post chelation ferritin with TSH levels; they showed a linear correlation ( Pearson coefficient of .836). Conclusion: Serum ferritin and TSH estimation in prechelation and postchelation periods give an estimate of iron overload with effect of chelation on it. Both levels decrease post chelation presenting a linear correlation between the two.

Keywords: Thalassemia; iron; chelation; ferritin; TSH.

Introduction: Thomas Cooley and Lee described the homozygous or compoundheterozygous state for a recessive Mendelian disorder not confined to the Mediterranean, But occurring widely throughout tropical countries. In the past 20 years, the two important Forms of this disorder, α- and β-thalassemia, resulting from the defective synthesis of the α-and β-globin chains of hemoglobin, respectively, have been recognized as the most common monogenic diseases in humans.¹,²,⁶

Under physiologic conditions, the concentration of iron in the human body is carefully regulated and normally maintained at approximately 40 mg iron/kg body weight in women and approximately 50 mg iron/kg body weight in men, distributed among functional, transport and storage components.²,³,⁷

Chronic blood transfusion is associated with many untoward complications like blood borne infections, isoimmunisation, febrile reactions and iron overload. Iron overload causes serum ferritin level to be raised in thalassemia. A single transfusion of two units of packed RBCs is about equal to a 1 to 2 year intake of iron. There are no mechanisms for increasing the excretion of iron beyond normal daily losses. Iron thus rapidly accumulates in chronically transfused patients. Common clinical complaints in iron overload include lethargy, weight loss, change in skin color, loss of libido abdominal pain and joint pain.⁵,⁷,⁸

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Iron accumulation in β-Thalassemia major patients depends upon the frequency of blood transfusions. 4 units of blood will contain around 1 gm of iron. Signs of clinical toxicity become apparent, when body iron reaches 400 to 1000mg/kg body weight. Signs of iron overload can be usually seen after 10-12 transfusions. In addition to the iron administered to blood transfusion, a hyperactive bone marrow will favour increased intestinal iron absorption that will contribute, although marginally to the total body load. The determination of ferritin is a suitable method for ascertaining the iron metabolism situation and it also reflects the thyroid status.5,7,9,11

Since serum ferritin level directly reflects the body iron status in the normal human subject, it is used as a routine test in the diagnosis of iron overload and monitoring the response to treatment. However, serum iron concentration is increased in some diseases even when the body iron stores are within normal limits, as in acute and chronic liver disease, malignancies, infections and megaloblastic anemia. This increased body iron can damage vital organs like heart, pituitary, thyroid, pancreas etc., causing morbidity and mortality.6,7,25,36

Hypothyroism is one of the most common endocrine complications in thalassaemias. Serum ferritin estimation reflects serum T.S.H. level. In present study, Deferasirox (oral iron chelating agent) is used for chelation. Iron chelation therapy helps in the binding of toxic non-transferrin bound iron in the plasma and the removal of iron from the body. It is clear that certain symptoms of iron overload such as cardiac arrhythmia and heart failure, can be improved well before local tissue levels of iron have decreased by the continual presence of aelator in the plasma.12,13,19,22,23,33

Chelation therapy should be started after one year of chronic transfusion. Ferritin represent a prognostic marker for thalassaemic patients and predictive factor for progression to thyroid dysfunction. Intensive chelation therapy allows prevention and reversibility of thyroid complication. The combination of blood transfusion and chelation therapy is import-tant in Indian context as it helps to identify the candidates of iron overloads and spare them from psycho somatic burdens of iron overload complication which compromise their life quality and expectancy.14,16,19,24,25,34,35

Aims & objectives: The main aim of the study was to know the effect of iron chelation in transfusion dependant thalassemia major patients with respect to serum ferritin, serum T.S.H. level before and after therapy. To correlate the level of post-chelation serum ferritin with the level of post-chelation serum T.S.H. in thalassemia major patients undergoing iron chelation therapy.

Materials & methods: The study was carried out amongst the detected thalassemia major patients who attended outdoor of a tertiary care centre undergoing iron chelation therapy (500 patients); taking note of the inclusion and exclusion criteria. Thalassemic patients selected were more than six years age, on regular blood transfusion with packed red blood cells for 1-3 months and all receiving iron chelation therapy. Those less than six years of age were excluded. The study was hospital based analytical descriptive one. Detailed history taken from patients with relevant clinical examination, and estimation of serum ferritin, TSH done. Ferritin estimation done by solid phase direct sandwich Elisa method (Benesphera Elisa machine). The standard samples and controls added into the selected wells coated with antiferritin antibody and incubated with 100 μl of incubation buffer. Ferritin in the standard controls, and serum bind to anti-ferritin antibody on the wells. Unbound protein washed off by wash buffer. Antiferritin HRP conjugated detection antibody added which then binds to ferritin. Unbound HRP washed off by wash buffer. Upon addition of substrates the intensity of colour was proportional to the ferritin concentration in sample. Ferritin was estimated by relating the colour intensity; absorbance on ELISA reader at 450 nm read within 15 minutes of adding the stopping solution. Expected values for this machine were 20-350 ng/ml in males and 10-200 ng/ml in females. TSH done by a one step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA) using a table top automated immunoanalyser (Vidas). 200 μl of sample transferred into well containing anti TSH antibody labelled with alkaline phosphatase conjugate and all steps are carried by SPR (solid phase receptacle serving as solid phase and pipetting device).

Post washing unbound components, during the final step, the substrate (4-methyl-umbelliferyl phosphate) got catalytically hydrolysed by the conjugate enzyme into a fluorescent product (4-methyl-umbelliferone) whose fluorescence measured at 450 nm. The intensity of fluorescence
Among the 500 participants, the mean post chelation ferritin level was 2168.80 ng/ml with a standard deviation of 1335.895 and mean post chelation TSH level was 4.5182 µIU/ml with a standard deviation of 4.76410.(Table :2)

**. Correlation is significant at the 0.01 level (2-tailed). While correlating post-chelation ferritin and T.S.H levels; Pearson correlation came as 0.836 which is statistically significant indicating a linear correlation between post-chelation ferritin and post-chelation T.S.H levels. (Table:4)

Discussion:
For many years, as thalassemia major patients suffer from moderate to severe anemia, therapy was limited to regular blood transfusion, mainly packed red blood cells aiming to keep the Hb level within 9 – 10 gm/dL to avoid morbidity related to excessive medullary and extramedullary erythropoiesis causing bony deformities, enormous hepato-splenomegaly and other complications followed by premature death.14,15,18,19

On the contrary, regular blood transfusion for several years resulting accumulation of iron, if untreated, cause considerable morbidity and ultimately leads to death. In addition, a hyperactive bone marrow in thalassemia increases the intestinal iron absorption. Iron balance in the body is primarily achieved by control of absorption rather than control of excretion. The total average daily loss of iron is about 1.0 mg/day in normal adult men and non – menstruating females.
Table 3: Paired t test: Pre and post chelation ferritin; pre and post chelation TSH

<table>
<thead>
<tr>
<th>Paired differences</th>
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<th>Paired differences</th>
<th>Paired differences</th>
<th>Paired differences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Standard deviation</td>
<td>Standard error mean</td>
<td>95% confidence interval of diff.</td>
<td>T</td>
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<tr>
<td></td>
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<td>Lower</td>
<td>Upper</td>
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<tr>
<td>Prechelation Ferritin</td>
<td>Postchelation Ferritin</td>
<td>826.978</td>
<td>668.814</td>
<td>29.910</td>
</tr>
<tr>
<td>Prechelation TSH</td>
<td>Postchelation TSH</td>
<td>.55200</td>
<td>3.05959</td>
<td>0.13683</td>
</tr>
</tbody>
</table>

Pair t-test in transfusion dependant thalassemia major patients with respect to Serum ferritin and T.S.H. level before and after chelation therapy shows 2-tailed p=0.000 and p being <0.05 presents it as statistically very significant. t>3 is also significant. ( Table : 3 )

women but menstruating women, in addition, loose highly variable amount of iron.16,17,18,19,36

The two main sources of chelatable iron are (a) the intracellular liable pool, derived from lysosomal catabolism of ferritin and from transferrin – and non – transferring – bound iron and (b) the iron derived from red cells catabolism in macrophages. To prevent hemosiderosis iron needs to be chelated and excreted. Thechelators are presently in use: desferoxamine (DFO), deferiprone and deferasirox.20,22,23,25,33

Detoxification of excess iron is probably the most important function of chelation therapy. Chelation therapy should be started after about one year of chronic blood transfusions. This correlates with a serum ferritin of approximately 1,000 ng/mL. While the standard recommendations have been to maintain a ferritin between 1,000 and 2,500 ng/mL, several programs are aiming to maintain serum ferritin at 500 ng/mL in adult patients. Liver and bone marrow biopsy are as such invasive procedures and thus cannot be used for frequent assessment of iron overload or for screening purposes. Patients with thyroid dysfunction were characterized by higher ferritin when compared with patients without thyroid disorders. Past works show the degree of correlation with serum ferritin and serum T.S.H. in patients of thalassemia major undergoing chelation therapy.20,22,23,25,33,35

Study by Malik Zeb Khan et al. (2014) shows serum ferritin used for efficient monitoring for iron chelation therapy and is suitable for ascertaining the state of iron metabolism. Study includes 50 thalassemia patients and tests done by electrochemiluminescence immune-assay technology (ECLIA) on cobase 411 Roche special immune assay analyzer. In their study, mean prechelation ferritin was 3467.97 ng/ml and mean postchelation ferritin was 2455.44ng/ml with a p value of 0.000 (where p< 0.05 considered significant)28

(8) A study by Chetna Jain et al. (2015) shows levels of Hb increased and serum ferritin decreased after chelation therapy. Study includes 150 thalassemia major patients using Immunoassay Analyzer Maglumi-1000 by chemiluminiscence method. In their study, mean prechelation ferritin was 2300 ng/ml and postchelation ferritin was 1300 ng/ml.29

Study by Hashemi et al. (2011) found no correlation between serum T.S.H. and serum ferritin. Here p-value = 0.99, p>0.05, statistically not significant.30 Study by Valerio Chirico et al. (2013) shows ferritin as a prognostic marker for thalassemia major patients and a predictive factor for progression to thyroid dysfunction. Intensive chelation therapy allowsthe prevention and reversibility of thyroid complication. The study comprises 72 thalassemia major patients under iron chelation therapy. Results shows:- p-value= 0.007 which is statistically significant. (p<0.05)31

The present study comprising of 500 patients, shows p=0.000 (p<0.05) and Karl Pearson coefficient−0.836. Correlation is significant at the 0.01 level (2-tailed). Serum ferritin bears a positive linear relationship with serum T.S.H. in post chelation state. Regarding
effect of chelation on serum ferritin; present study results show similarity with Malik Zab Khan et al. (2014). Here, both studies show decrease of serum ferritin after chelation. Chetna Jain et al. (2015) bears resemblance with present study result. Both study results show decrease of serum ferritin after chelation. Present study results bears no resemblance with Hashemiet al. (2011). His study found no correlation between post chelation serum T.S.H. and serum ferritin. Present study shows post chelation ferritin bears positive linear correlation with serum T.S.H.Even study by Nijagna N et al (2015)found no linear correlation of serum ferritin with thyroid dysfunction. Both Valerio Chirico et al. (2013) and present study show similar results where post chelation ferritin bears positive linear correlation with post chelation serum T.S.H. In present study; mean pre chelation serum ferritin and serum T.S.H. of the five hundred (n=500) thalassemia major patients are – 2995 ng/ml & 5.07 micro I.U/ml respectively. This shows patients have iron loads which need chelation [cut off values for chelation is 1000 ng/ml] and have hypothyroidism [upper cut off values for T.S.H. at that age group is 4.3 u IU/ml]. After chelation therapy of the same five hundred patients; mean serum ferritin decreased (2168 ng/ml) with decrease in mean serum T.S.H. (4.5micro IU/ml). So, chelation has a positive role on ferritin and T.S.H.status causing decreased body iron load & toxicity and preventing or improving hypothyroidism . Post chelation ferritin shows a positive linear correlation with post chelation serum T.S.H. as evident from the Pearson Correlation Coefficient of 0.836. From the values of post chelation ferritin &T.S.H. of the above five hundred patients, serum ferritin act as a predictive factor for progression to thyroid dysfunction. Serial estimation of serum ferritin in thalassemia major patients reflects the serum T.S.H. which helps in the diagnosis and prognosis of hypothyroidism of thalassemic major patients as most of the patients are suffering from hypothyroidism with no clinical evidence which is very much useful specially in a developing country like India, with high prevalence of thalassemic patients.

**Conclusion:** Iron chelation and estimation of serum ferritin with TSH assay is important in Indian context as they help to identify the candidates of iron overload & hypothyroidism and spare them from psychosomatic burden of iron overload complication which compromise their life quality and expectancy.

**Conflict of interest:** None.

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**Ethical clearance:** Permission granted by Ethical committee of institution.

**Author’s contribution:** All authors participated equally in Data collection, Statistical analysis and Writing and submission of manuscript.

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