

# Study on Association of Serum Ferritin With Thyroid Profile And Oral Glucose Tolerance Test in Thalassemia Major Children

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## ABSTRACT

**Introduction:** The free iron and haemosiderosis-induced damage of the endocrine glands cause endocrinopathies such as abnormal glucose tolerance and hypothyroidism in transfusion - dependent beta-thalassemia major patients. Our objective was to study the association of serum ferritin level with thyroid dysfunctions; abnormal blood glucose tolerance and to see if they appear in the earlier period of life.

**Methods:** This cross-sectional study was done among thalassemia major children of two to 12 years in a tertiary care hospital, Kolkata, India. A pre-designed proforma was filled. Serum ferritin, fT4, TSH level, and oral glucose tolerance test (OGTT) were measured at presentation and noted in proforma.

**Results:** A total of 80 thalassemic children were studied. Fiftieth percentile cut off value (1414 ng/ml) of serum ferritin was found to be significant with associated variables like normal fT4, TSH, and OGTT. Out of all study subjects, 39 (51.3%) of normal fT4, 39 (54.9%) of normal TSH and 39 (52.0%) of normal OGTT had ferritin < 50<sup>th</sup> percentile (P < 0.05). Nine (11.3%) children had abnormal thyroid profiles and five (6.3%) children had abnormal OGTT having ferritin > 2000 ng/ml. At a cut off value of ferritin level > 1414 ng/ml, fT4, TSH and OGTT showed significant abnormality (p < 0.05 with df 1).

**Conclusions:** Ferritin is a good indirect marker to assess the risk of endocrine abnormality in thalassemic children. Frequent monitoring should be done once ferritin level crosses 1000 ng/ml. This will help in early detection and timely management of thalassemia related endocrinopathies.

**Keywords:** endocrinopathies; serum ferritin; Thalassemia major



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## INTRODUCTION

Thalassemia refers to a spectrum of inherited disorders of haemoglobin synthesis characterised by reduced or lack production of one or more globin chains.<sup>1</sup> Beta thalassemia is due to impaired production of a beta-globin chain, leading to a relative excess of the alpha-globin chain. An excess alpha-globin chain is incapable of forming soluble tetramers on its own and precipitates within the cell leading to damage of the red cell membrane and reduced red cell survival.

Children with thalassemia major receive regular blood transfusions to maintain their normal haemoglobin. Regular blood transfusion and increased absorption of iron due to ineffective erythropoiesis lead to excess iron in the body. Accumulation of iron in different tissue causes organ damage affecting mainly endocrine glands, heart and liver.<sup>2</sup> A complication of iron overload includes endocrine complications {growth retardation, failure of sexual maturation, diabetes mellitus (DM) and insufficiency of the parathyroid, thyroid, pituitary and less commonly adrenal

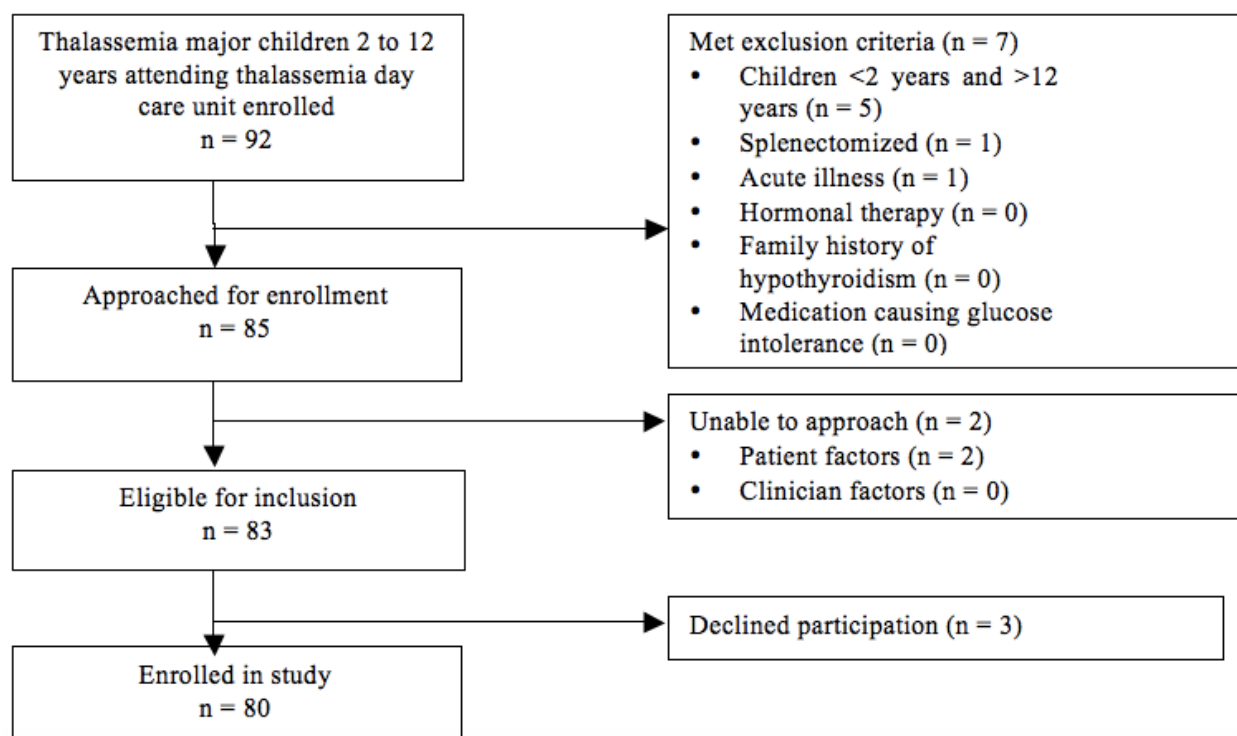
glands}, dilated cardiomyopathy, liver fibrosis, and cirrhosis are other common complications.

The widespread form of thyroid dysfunction seen in thalassemia is primary hypothyroidism, caused by abnormalities of the thyroid gland which result in insufficient production of thyroid hormones. Central hypothyroidism caused by decreased secretion of TSH from the anterior pituitary gland or by decreased secretion of TRH from the hypothalamus is less common.

Endocrine dysfunction affects virtually all the glands. Up to 14% may develop Insulin-dependent Diabetes Mellitus.<sup>3</sup> Even those without diabetes have impaired insulin secretion. DM in patients receiving hyper transfusion for thalassemia major is usually attributed to damage to beta cells resulting in insulin deficiency secondary to iron deposition in pancreatic islet cells. Reports of hyperinsulinemia with abnormal glucose tolerance tests may suggest a role of insulin resistance.<sup>4</sup>

## METHODS

It was cross-sectional observational hospital-based study done in thalassemia major children of two



**Figure 1.** Patient screening and enrolment

years to 12 years attending thalassemia day care unit of a tertiary care hospital, Kolkata, India. The study was conducted over a period of one year, from February 2018 to March 2019 after obtaining the requisite permission of the Ethics Committee of our institute. A total of 80 thalassemic children were studied. The included children were of age range > two years to < 12 years and diagnosed thalassemia major children by HPLC and were transfusion-dependent. Those children < two years and > 12 years were excluded. The children who were splenectomised, had acute illness or were under hormonal therapy, with family history of hypothyroidism and did not give consent were excluded.

After obtaining detailed informed consent, the children were undertaken for study. A pre-designed proforma was filled which included a detailed history, systemic examination, investigations. The parameters (Age, Sex, Serum ferritin, fT4, TSH level and oral glucose tolerance test) were measured at presentation and noted in proforma.

Venous blood was drawn for investigation (for HPLC, ferritin, fT4, TSH, and OGTT) under full aseptic procedure of which 5 ml was collected in EDTA anticoagulant for HPLC, 3 ml blood in a clotted vial for serum ferritin, fT4 and TSH. In this study, we used the World Health Organisation's definition of diabetes. An oral glucose tolerance test (OGTT) is performed in the morning after an overnight fast, as recommended by the National Diabetes Data Group. Glucose is ingested in a dose of 1.75 g/kg up to a maximum of 75 g and blood samples (2 ml in Grey topped tube) will be obtained at 0 and 120 min for measurement of plasma glucose (by glucose oxidase method). Two hour value > 140 mg/dl will be considered as an abnormal or impaired glucose tolerance test.

fT4, TSH and ferritin levels were studied in basal blood samples employing Electrochemiluminescence Immunoassay (ECLIA) method using the Roche Diagnostic E 170 Auto analyser. (Reference range for fT4 (1 – 12 yr): 0.8 – 2.2 ng/dl; TSH (1 – 6 yr): 0.85 – 6.5 micro I.U. /ml, (6 – 12 yr): 0.28 – 4.3 micro I.U. /ml).

The data were entered into the Microsoft excel enterprise 2007 spreadsheet. The analysis of the available data was done by using IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp. 2013 and Graph Pad Prism version 5. The categorical variables were analysed with the help of required non-parametric test like Pearson Chi-Square test and Fisher Exact test where ever necessary. Continuous variables were analysed with the help of required parametric tests like independent t-tests. The efficacy ratio was calculated for the outcome measures by using the CAT Maker software. The level of significance was considered as 95% confidence interval. So p-value  $\leq 0.05$  was considered statistically significant.

## RESULTS

We divided our patients into three groups based on the ferritin level. First group (Ferritin level < 1000 ng/ml) comprises of 19 patients (Male 11 and Female 8). Majority (55%) of the patients came under second group (Ferritin level 1000-2000 ng/ml) - male 17 and female 27. The last group with ferritin level (> 2000 ng/ml) comprises of 17 patients (male 12 and female 5). We also divided our study children into two groups according to their age - (i) Age  $\leq 6$ yr and (ii) Age > 6 yr. For statistical calculation, percentile-based cut off analysis has been performed. 25<sup>th</sup> percentile cut off value of serum ferritin was found to be non-significant with association variables like normal free T4, normal TSH and normal oral GTT but 50<sup>th</sup> percentile cutoff (1414 ng/ml) were significant. Both intra-group and inter-group comparison of numerical variables was done by Independent t-test. Whereas in the case of categorical variables, intra-groups comparison were performed using Chi-square test/ Fisher's exact test according to the situations. Of total boys (n = 40) 22 (55.0%) were boys of up to six years of age and 18 were older than six years. Among girls, 22 (55%) were up to six years and 18 (45%) were older than six years. Age group and gender were independently distributed in this study (p = 1.000). Serum ferritin level and TSH levels were significantly correlated with age and serum-free T4 level did not correlate with age. Gender of the study subjects when compared with their serum ferritin level, serum-free T4 level and serum TSH level applying

**Table 1.** Association between normal serum free T4, normal serum TSH, OGTT and serum ferritin level (as per cut off used in this study) (N=80)

Item		Ferritin Range		Total	Test statistics
		< 50th percentile	> 50th percentile		
Normal FT4	Yes	39 (51.3%)	37 (48.7%)	76 (100.0%)	T value 4.005 Df 1 p < 0.05 (0.045)
	No	0 (0.0%)	4 (100.0%)	4 (100.0%)	
Normal TSH	Yes	39 (54.9%)	32 (45.1%)	71 (100.0%)	T value 9.646 Df 1 p < 0.05 (0.002)
	No	0 (0.0%)	9 (100.0%)	9 (100.0%)	
OGTT	Abnormal	0 (0.0%)	5 (100.0%)	5 (100.0%)	T value 9.646 Df 1 p < 0.05 (0.002)
	Normal	39 (52.0%)	36 (48.0%)	75 (100.0%)	

\*Parenthesis indicate percentages (%)

Independent t-test it was found that none of the above variables were dependent on gender. The oral glucose tolerance test had a significant correlation with ferritin, fT4 and TSH. fT4 was significantly associated with ferritin and TSH value; TSH was significantly associated with ferritin and fT4 value.

Out of all study subjects, 39 (51.3%) of those with normal serum free T4 level had a serum ferritin level less than cut off value of 1414 ng/ml or <50th percentile (p = 0.045). Thirty nine (54.9%) of those with normal TSH levels had a serum ferritin level less than cut off value of 1414 ng/ml or < 50th percentile (p = 0.002). 39 (52.0%) of normal OGTT had serum ferritin level less than cut off value of 1414 ng/ml or <50th percentile (p = 0.002) Table 1. Nine (11.3%) children had abnormal thyroid profile Table 2 and 5 (6.3% of total study children) children had abnormal OGTT Table 3 and all of them had ferritin > 2000 ng/ml. Abnormal thyroid

profile and abnormal OGTT had a mean age of 11 ± 1 yrs.

Lastly, when three outcome variables (fT4, TSH and OGTT) were compared together with serum ferritin level at a cut off value of 1414, there was a significant risk of abnormal development of any of the three parameters (p < 0.05 with df 1).

## DISCUSSION

The present study was aimed to find out the association of serum ferritin with thyroid profile and oral glucose tolerance test in thalassemic children. We included 80 thalassemia major children admitted for blood transfusion in our hospital, who met the inclusion criteria. In our study, nine (11.3%) children had abnormal thyroid profile, which is supported by other studies.<sup>5-7</sup> But a study in India by Ghosh S et al. in 2017 also detected 30% hypothyroidism of which 17.5% had subclinical hypothyroidism, 11.25% had primary

**Table 2.** Ferritin level and thyroid profile

Item		Thyroid Function Status		Total	Test statistics
		Hypothyroid	Euthyroid		
Ferritin category	< 1000	0 (0.0%)	19 (100.0%)	19 (100.0%)	X <sup>2</sup> 37.581 Df 2 p < 0.05 (0.000)
	1000 - 2000	0 (0.0%)	44 (100.0%)	44 (100.0%)	
	> 2000	9 (52.9%)	8 (47.1%)	17 (100.0%)	
Total		9 (11.3%)	71 (88.8%)	80 (100.0%)	

\*Parenthesis indicate percentages (%)



Table 3. Ferritin Level &amp; OGTT

Item	OGTT		Total	Test Statistics
	Abnormal	Normal		
Ferritin Range	< 50th percentile	0 (0.0%)	39 (100.0%)	$\chi^2$ value 5.073 Df=1 p <0.05 (0.024)
	> 50th percentile	5 (12.2%)	36 (87.8%)	
Total	5 (6.3%)	75 (93.8%)	80 (100.0%)	

hypothyroidism and 1.25% had secondary hypothyroidism.<sup>8</sup> Another study in London by Grundy RG. in 1994 on 18 transfusion-dependent beta thalassaemic children showed a prevalence of abnormal thyroid function of 11%. In the study, they could not identify any particular risk factor. But their children were receiving hyper transfusion with suboptimal iron chelation therapy. It may explain the increased prevalence of hypothyroidism due to more iron overload.<sup>9</sup> All the patients with abnormal thyroid profiles in our study had primary hypothyroidism. A preponderance of primary hypothyroidism is supported by other studies.<sup>9,10,11</sup> No patient had any clinical signs and symptoms of hypothyroidism. This finding again was supported by several other studies, which were conducted, by Abdel-Rajek AR et al. in 2013 and Landau H et al. in 1993.<sup>7,10</sup> The mean age for the patient showing abnormal thyroid profile was  $11 \pm 1$  yrs and we did not get any such patient below 10 years of age. However, a study in India in the year 2013 to 2015 by Upadya SH et al. showed the occurrence of hypothyroidism even in the first decade of life.<sup>11</sup> Other studies like Gulati R et al. in 2000 and Abdel-Rajek AR et al. in 2013 also support the early occurrence of hypothyroidism at an earlier age.<sup>6,7</sup> This may be due to the small sample size in our study.

The positive association of increased frequency of hypothyroidism with increased serum ferritin level is documented in many research works.<sup>7,11</sup> We also got the same result (p-value < 0.005). In our study, the average serum ferritin level in abnormal fT4 and abnormal TSH value is 3348.5 ng/ml and 3338.3 ng/ml respectively. All the children with abnormal thyroid profiles had serum ferritin value > 2000 ng/ml. 77.8% of the boys and 22.2% of the girls with ferritin level > 2000 ng/ml showed

abnormal thyroid profile. In our study children, the 50th percentile of serum ferritin value was 1414 ng/ml. It was found that the serum ferritin level was significantly associated with serum-free T4 level (negative association) at the value of more than 1414 ng/ml. There is a risk of development of hypothyroidism (low fT4) in subjects with a high serum ferritin level of more than 1414 ng/ml (T value 4.005 with p = 0.045 at df 1). There was a decrease in fT4 level with increasing serum ferritin level, extremely high level of serum ferritin when separated from a relatively lower level, it became statistically significant. We also found that the serum ferritin level was significantly associated with serum TSH level (Positive association) at the value of more than 1414 ng/ml. There was a risk of development of hypothyroidism (high TSH) in subjects with a high serum ferritin level of more than 1414 ng/ml (T value 9.646 with p=0.002 at df 1).

Regarding oral GTT, we had five (6.25% of total study children) children with an abnormal result. This finding matches with other studies.<sup>5,6</sup> We noticed that 39 (52.0%) of those with normal OGTT had serum ferritin level less than cut off value of 1414 ng/ml and risk of development of abnormal OGTT in subjects with high serum ferritin level of > 1414 ng/ml was statistically significant (T value 9.646 with p-value of 0.002 at df 1). This finding is supported by a study done by Chern PS et al. in Taiwan in the year 2001.<sup>12</sup> A study done by Robabeh Ghergherehchi et al, in Iran in the year 2013<sup>13</sup> which concluded abnormal glucose metabolism is common in  $\beta$  - thalassemia major patients with chelation therapy and multiple transfusions which are attributable to impaired  $\beta$  cells' function and increased insulin resistance. In our study, the average ferritin level among children

with abnormal OGTT was 3522.6 ng/ml. All the children with abnormal OGTT had serum ferritin value > 2000 ng/ml. In our study we had, 12.5% of the boys showed abnormal glucose tolerance, which was statistical significant. When three outcome variables (fT4, TSH and OGTT) were compared together with serum ferritin level at a cut off value of 1414, there was a significant risk of abnormal development of any of the three parameters ( $p < 0.05$  with df 1). This finding is corroborative with a study done by Gulati R et al. in 2000 in India.<sup>6</sup> They got 33% of their study children (preadolescent group N = 54) having at least one endocrine deficiency.

The limitations of our study are small study sample, short duration of the study; measurement of serum ferritin was not uniform in study children. The duration of disease, frequency of blood transfusion and effect of chelation therapy were not kept in consideration. The effect of the dietary pattern was not considered.

## CONCLUSIONS

Endocrinopathy is a common morbidity in children with thalassemia major in our society.

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Determination of serum ferritin at regular intervals is necessary to detect any disturbance in thyroid function and OGTT as well as to establish an appropriate protocol for investigation and treatment. Ferritin is a good indirect marker to assess the risk of endocrine abnormality in thalassemic children. Thyroid profile and OGTT should be regularly monitored from time to time to detect these disorders and treat them appropriately with proper chelation. We should be more cautious and more frequent monitoring should be done once ferritin level crosses 1000 ng/ml. This will help in early detection and timely management of thalassemia related endocrinopathies and thus in broader aspect will help to improve quality of life of thalassemic children.

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