



## A STUDY OF CORRELATION BETWEEN THYROID HORMONE PROFILE (SERUM FREE T4 AND TSH) AND SERUM FERRITIN LEVEL IN TRANSFUSION DEPENDENT $\beta$ -THALASSEMIC PATIENTS

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### ABSTRACT

Transfusion related iron overload is the primary therapeutic complication in  $\beta$ thalassemia major patients. Iron deposition in various endocrinal glands is responsible for the hormonal derangements. One of the reported endocrinopathy is hypothyroidism. The present study was aimed to evaluate thyroid hormone profiles and serum ferritin level in transfusion dependent  $\beta$  thallemic patients and to find out whether there was any significant correlation between them. Serum ferritin, free T4 and TSH were measured by ELISA method in diagnosed transfusion dependent beta thalassemia patients. Serum ferritin level ( $1975.11 \pm 687.28$  ng/ml) in patients was higher than normal reference value whereas free T4 level ( $0.90 \pm 0.26$  ng/dl) was nearer to lower range and TSH level ( $5.82 \pm 2.08$ ) was nearer to higher range of normal reference value. This study demonstrated significant positive correlation between TSH and serum ferritin level ( $r=0.694$ ) and significant negative correlation between fT4 and Ferritin ( $r=-0.731$ ) (P value  $<.001$ ). This study demonstrated that 24 patients (30%) of total thallemic patients (n=80) had one kind of thyroid dysfunction. Ferritin levels were significantly high in patients with thyroid dysfunction ( $2667.46 \pm 536.24$  ng/ml) than euthyroid patients ( $1678.39 \pm 509.38$  ng/ml) (p $<.001$ ). High Serum ferritin level in beta thallemic patients reflects iron overload in body. High Ferritin level was significantly related to thyroid hormone profile. Deposition of iron in thyroid gland and other tissues may cause oxidative damage affecting thyroid hormones synthesis or its peripheral conversion. Thus high serum ferritin level may indicate the need of thyroid hormone assessment for early detection and initiation of treatment of thyroid disorders in transfusion dependent  $\beta$ -Thallemia patients.

### Keywords:

### INTRODUCTION:

Beta-thalassemia is one of most common autosomal recessive disorders worldwide.  $\beta$ -thalassemia is the commonest single-gene disorder in the Indian population(1). Ten percent of the total world thallemics are born in India every year (2). Certain communities in India, like Sindhis, Gujratis, Punjabis, and Bengalis, are more commonly affected with beta thalassemia, the incidence varying from 1 to 17%(3).  $\beta$  thalassemia arises from deficient  $\beta$  globin chain production.  $\beta$  thalassemia major is a very serious blood disorder since individuals with it are unable to make enough healthy red blood cells and depend on blood transfusion throughout their life(4). The combination of blood transfusion and chelation therapy has dramatically prolonged the life expectancy of these patients, thus transforming thalassaemia from a rapidly fatal disease of childhood to a chronic disease compatible with a prolonged life(5). Frequent blood transfusions can lead to iron overload which may result in several endocrine complications. Hemosiderosis of various endocrine glands including the thyroid gland has been documented histologically in chronically transfused patients including thallemics. Iron deposition in various endocrinal glands is responsible for the hormonal derangements(6-8). One of the reported endocrinopathy is hypothyroidism. Thyroid profiles can be assessed by measuring serum free T4(fT4) and TSH levels. Diagnoses of thyroid dysfunction were based on the following:

1. Primary hypothyroidism is diagnosed when Free T4 is  $<0.8$  ng/dl, and TSH is  $>6.16$  mIU/ml

2. Subclinical hypothyroidism is diagnosed when Free T4 is normal ( $0.8 - 2.0$  ng/dl), and TSH is  $>6.16$  mIU/ml

3. Central hypothyroidism is diagnosed when Free T4 is  $<0.8$  ng/dl and TSH is low or normal.

4. Hyperthyroidism is diagnosed when Free T4  $> 2$  ng/dl and TSH is normal or low

Serum ferritin is one of the storage form of iron in the body. Minute quantities of ferritin are present in the serum in concentrations roughly proportional to total body-iron stores. Increased body iron overload may be reflected by increased serum ferritin level. Serum ferritin levels widely and commonly used to assess iron overload in beta thalassemia patients.

### Materials and methods:

The present study is a hospital based cross sectional study involving Department of Biochemistry and Thallemia Control unit under the Department of Pathology.

### Study Population: cases and controls

For our study we have selected a group of 80 patients with beta thalassemia major treated with blood transfusions attending Thallemia Control unit of our College and Hospital for periodical follow up.

The study was approved by the Institutional Ethical Committee. Informed consent was taken from every participant or their guardian as applicable.

### Inclusion criteria:

Diagnosed  $\beta$  thalassemia patients male or female receiving regular blood transfusions for more than one year.

**Exclusion criteria:**

Patients with history of congenital hypothyroidism or other thyroid diseases, acute infections and inflammatory conditions, any psychiatric illness on antipsychotic medicines (Lithium etc.) ,any malignancy, Chronic liver diseases, terminally and critically ill patients were excluded from study.

**Collection and storage of sample:**

Fasting blood samples for free T4, TSH and Ferritin were collected from patients and stored at  $-20^{\circ}\text{C}$  till further estimation. Samples were thawed to room temperature before every assay and repeated thawing was avoided.

**Estimation of test parameters:**

For estimating all the parameters, ELISA (Enzyme linked immunosorbent assay) was done with commercially available AccuBind ELISA reagent kit for serum free T4, TSH and Ferritin. Standard curve was performed for each assay using norms provided by the manufacturers.

**Table1: Method, company, Lot no. Unit and Normal range of Company of Test Parameters**

Test	Method	Product/Company	Lot No.	Unit	Normal Range
TSH	ELISA	AccuBind	EIA-3K1D16H241	$\mu\text{IU/ml}$	0.39-6.16
Free T4	ELISA	AccuBind	EIA-12K5B4H	ng/dl	0.8-2.0
Ferritin	ELISA	AccuBind	EIA-28K2A6	ng/ml	Males: 16-220 Females: 10-124

**Statistical Analysis:**

Calculations were performed using SPSS 20 software for Windows. Spearman’s rho correlation was used to determine correlation of serum FT<sub>4</sub> and TSH with ferritin level. Kruskal Wallis test was used to check if the differences of mean rank of ferritin varying significantly with different thyroid function groups .

**Results:**

This study has been designed to to determine whether any significant correlation between thyroid profiles and serum ferritin level in transfusion dependent  $\beta$  thalassaemic patient is present or not. For this 80 subjects were selected on the basis of inclusion and exclusion criteria of the study.

Mean age of the patients was 6.9 years. Mean ferritin, TSH and free T4 value of patients were 1975 ng/ml, 5.82  $\mu\text{IU/ml}$ , 0.90ng/dl respectively (Table 2).

Spearman’s rho correlation demonstrated positive correlation between TSH and serum ferritin level ( $\rho=0.694$ ) and negative correlation between FT<sub>4</sub> and Ferritin ( $\rho=-0.731$ ) which were statistically significant (P value  $<.001$ ). That means TSH value increases and FT<sub>4</sub> value decreases with increased ferritin level (Table 3).

Among 80 patients 24 patients(30%) had one kind of thyroid dysfunction and rest 56 patients(70%) were euthyroid. 14 patients (17.5%) had subclinical hypothyroidism 9 patients (11.25%) had primary hypothyroidism and one patient(1.25%) had secondary hypothyroidism. No patient with hyperthyroidism was detected(Table 4).

Kruskal Wallis test demonstrated mean rank of serum ferritin in Euthyroid, sub clinical, primary and total thyroid dysfunction were 33.64, 63.21, 88.94 and 74.44. The mean rank of ferritin of thyroid dysfunction groups were significantly high than euthyroid group (pvalue $<.001$ ) (Table 5,6)

**Table 2: Table showing statistical summary of variables**

Parameter	Mean	SD	Median	Maximum	Minimum	N
TSH( $\mu\text{IU/ml}$ )	5.82	2.08	5.80	13.00	0.38	80
Free T <sub>4</sub> (ng/dl)	0.90	0.26	0.91	1.94	0.17	80
Ferritin (ng/ml)	1975.11	687.28	1694	3652	802	80

**Table3: Correlation of Serum ferritin with other parameters**

Parameters	Spearman’s rank correlation coefficient ( $r_s$ )	‘p’ value	Significance
Free T <sub>4</sub>	-0.731	$<.001$	Significant
TSH	0.694	$<.001$	Significant

\*\*Correlation is significant at the level 0.01(2 tailed)

**Table 4 : Table showing distribution of thyroid function status in patients**

Thyroid Status	No. of Patients	percentage
Euthyroid	56	70
Subclinical Hypothyroidism	14	17.50
Primary hypothyroidism	9	11.25
Secondary Hypothyroidism	1	1.25
Total	80	100

**Table 5: Mean Ranks of ferritin and mean ferritin in different group of thyroid function**

Thyroid Function	Mean Rank of Ferritin	Mean Value of Ferritin(ng/ml)
Euthyroid (N=56)	33.64	1678.39
Subclinical Hypothyroidism(N=14)	63.21	2377.28
Primary Hypothyroidism (N=9)	88.94	3022.22
Total Thyroid Dysfunction(N=24)	74.44	2667.46

**Table6: Chi-Square test to show any significant relationship between Ferritin level and different group of thyroid function.**

Test Statistics <sup>a,b</sup>	
	Ferritin
Chi-Square	50.416
df	3
Asymp. Sig.	.000
a. Kruskal Wallis Test	
b. Grouping Variable: Group	

Eighty transfusion dependent beta thalassemia patients were evaluated in this study. The results showed that in totally 24 patients (30%) had one kind of thyroid dysfunction. There was significant positive correlation between serum TSH and ferritin and significant negative correlation between Ft4 and serum ferritin. The ferritin level was significantly high in hypothyroidism patients than euthyroid patients.

Aysegul Ugur Kurtoglu et al.in their study found found that 12.8% of beta thalassemia major patients had hypothyroidism(9).

Zervas et al. reported that 4% had hypothyroidism, and 12.5% had subclinical hypothyroidism. The results showed monitoring of blood transfusion decreased hypothyroidism(10).

Sara Ahmed Malik et. al demonstrated in their study that of the 70 cases of β-thalassaemia with an age range of 5-14 years (mean age 7.6 ± 2.5 years). Primary hypothyroidism was present in 18 (25.7%) patients(11).

Hashemi.A et.al in a cross sectional study demonstrated that fourteen patients (21%) had hypothyroidism mean age group is 10.3 years(12).

Gathwala Geeta et al conducted a similar case control study in Thalassaemia day care center, Department of Pediatrics, Pt. B.D. Sharma Postgraduate Institute of Medical Science, Rohtak, India with mean age (±SD) of thalassemia cases was 7.97 ± 2.83 years (range 3-13 years). The mean TSH level was significantly higher (p<0.01) in cases (3.56 ± 1.49 µg/dL) as compared to controls (2.31 ± 2.74 µg/dL). Among thalassemia cases 70% were euthyroid (n=35), 18% were compensated hypothyroid (n=9), 12% were uncompensated hypothyroid (n=6) and none was overt hypothyroid or hyperthyroid(13). Similarly in this present study among the thalassaemic patients 70% euthyroid and 30% patients of thyroid dysfunction were detected.

This present study demonstrated significant positive correlation between TSH , serum ferritin and significant negative correlation between Serum free T4, ferritin level (P value<.001) . Difference of serum ferritin level in euthyroid and other group of thyroid dysfunctions were significant (P value<.001). This is comparable with following studies.

Nashwa Mamdouh Samra et.al showed significant higher level of serum ferritin level in hypothyroid group as compared to euthyroid group (p value <0.001). Their study showed positive association of serum ferritin and TSH level and duration of transfusion (p value < 0.005) and showed that there were statistically significant positive association (p-value <0.001) between TSH and duration of transfusion(14).

Dr. Hussein Kadhem Abdul Hussein Al-Hakeim et.al in case control study indicated that the most significant positive correlations were present in serum TSH with Iron, ferritin, While T<sub>4</sub> hormone was correlated with ferritin( p value<0.05) (15).

Sara Ahmed Malik et.al demonstrated mean ferritin level was 3924 ± 1247ng/ml in hypothyroid and 3136 ± 1387ng/ml in euthyroid patients indicating a significant difference in mean serum ferritin levels between hypothyroid patients and others (p=0.037)(11).

**Discussions:**

Many mechanisms responsible for thyroid dysfunction in thalassemic patients have been suggested, however the exact mechanism is not known. It has been demonstrated that thyroid abnormalities in these patients are related to iron overload. The precise mechanism for which is not completely understood. High concentrations of labile plasma iron and labile cell iron are considered responsible in the formation of free radicals and the production of reactive oxygen species (ROS) which may lead to cell and organ damage. Organ siderosis (liver, cardiac and skeletal muscle, kidney) may affect specific receptors, which regulate thyroid hormone action and convert T4 to the bioactive T3(16).

Our study showed significant higher level of serum ferritin level in hypothyroid group as compared to euthyroid group (p value <0.001). Similar results were detected by Pirincioğlu et al., (17).

Our finding disagrees with Thuret et al., (18), Eschragi et al., (19) and Jaruratanasirikul et al., (20) as they found that incidence of hypothyroidism was not related to high level of serum ferritin level. These findings suggest possibility that spot serum ferritin sample may not be sufficient alone to determine the implication of chronic iron exposure in developing thyroid dysfunction. There is no doubt that iron overload has important role in thyroid and other endocrinal dysfunction in thalassemic patients, the non-significant difference in ferritin levels between hypothyroid and euthyroid group suggests that the damage of endocrine glands caused by chronic hypoxia due to prolonged anemia may be associated factor responsible for thyroid dysfunction.

Contrary to present study several studies have reported a lack of concordance of ferritin concentrations with the thyroid function status(21-23). This may be, in part, due to the fact that serum ferritin levels increase linearly with the transfusion load up to 100 units of transfused blood, but thereafter there is no simple relationship(24). Also, misleading ferritin levels can occur with chronic inflammatory disease(25) as well as vitamin C deficiency(26).

### **Conclusion:**

High serum ferritin level may indicate a risk for developing thyroid disorders and emphasize the need of thyroid hormone assessment in transfusion dependent beta thalassemia patients. Early recognition and prevention of these complications could improve the quality of life of these patients.

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