Original Article

Thalassemia

Serum Ferritin Level in Thalassemic Patients of 10-15 Years and its Relationship with Thyroid Function Tests

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ABSTRACT

Objective: To determine the prevalence of hypothyroidism and to evaluate any possible correlation between serum ferritin level and thyroid function tests in transfusion dependent beta-thalassemic patients.

Study Design: An observational and correlation study.

Place and Duration of Study: This study was conducted at DHQ hospital, Hilal-e-Ahmar hospital and Ali Zaib Foundation hospital Faisalabad from 15th of May 2011 to 15th of Jan 2012.

Materials and Methods: A total number of 90 thalassemia major patients, of 10-15 years of age, were included in this study. Serum levels of thyroxine, tri-iodothyronine, thyroid stimulating hormone were determined and correlated with serum ferritin level. Five milliliters of blood was drawn from each subject and thyroid profile (T_3, T_4 and TSH) and serum ferritin were determined. Pearson correlation coefficient test was applied to determine any correlation between serum ferritin level and other parameters.

Results: There was a weak negative correlation of Serum Ferritin with both Triiodothyronine and Thyroxine, but both correlations were statistically insignificant (p-value= 0.294 & 0.189 respectively). Serum Ferritin had a weak positive, but insignificant correlation with TSH. Hypothyroidism was detected in 15 patients (16.67%). Out of these 8 (8.89%) were having subclinical hypothyroidism, 5 (5.56%) mild hypothyroidism, 1 (1.11%) overt hypothyroidism and 1(1.11%) patient having secondary hypothyroidism.

Conclusion: High prevalence of hypothyroidism warrants regular screening of thyroid functions in thalassemic patients irrespective of their serum ferritin levels.

Key Words: Beta thalassemia, hypothyroidism, Ferritin.

INTRODUCTION

Inherited hemoglobin disorders are the most prevalent single gene defects in human beings across the globe[1], and thalassemia is the commonest in this group[2]. Beta-thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent beta (β) globin chain synthesis, resulting in reduced hemoglobin in red blood cells[3]. According to Thalassemia International Federation, about 2,00,000 patients of thalassemia major are alive and receiving treatment globally[3]. In Pakistan, gene frequency of β-thalassemia has been estimated to be 5-8% and about 8-10 million carriers[4] and is present in all ethnic groups[5].

Anemia in these patients is caused by hemolysis and ineffective erythropoiesis, which is characterized by enhanced apoptosis of the maturing nucleated erythroid cells[6.7]. Thalassemia treatment depends on regular blood transfusions but after about one year of transfusions, iron begins to accumulate in parenchymal tissues where it may cause substantial toxicity as compared to that in reticuloendothelial tissues[8].

Because there is no known mechanism to excrete excess iron from the body, repeated transfusions and poor compliance to therapy and chronicity of the disease lead to iron overload- related complications including endocrine dysfunctions[6]. Ferritin is the principal iron storage protein[9], and iron deposits in thalassemics, who have been receiving multiple blood transfusions, can exceed the storage and detoxifying capacity of ferritin, consequently free iron begins to accumulate in the tissues and blood[10].

Thyroid dysfunction in β-thalassemic patients has been reported ranging from a low prevalence of 0-12% to 10-35% in different cohorts[11]. Hypothyroidism is the second major endocrine complication resulting from hemosiderosis[12] Abnormal thyroid functions may be reversible at an early stage through intensive chelation therapy[13].

The aim of the present study was to investigate thyroid hormones and serum ferritin levels and to detect any correlation and presence of hypothyroidism in patients having high serum ferritin level.

MATERIALS AND METHODS

Study Design: The observational and correlation study was conducted on patients recruited from Thalassemia center of District Headquarters Hospital, Ali Zaib Blood Transfusion Services and Thalassemia center of Hilal-e-Ahmar Hospitals, Faisalabad. The study was conducted from 15th of May 2011 to 15th of Jan 2012 (eight months).

Ethical Considerations: Study was conducted as per Helsinki Declaration of Human Rights. Approval was obtained by Ethical Review Committee of Punjab Medical College, Faisalabad. Written informed consent was taken from each patient.

Methodology: Personal data was recorded on a proforma, specifically designed for the study. Patient's age, sex, and blood group etc. were recorded. Five milliliters of venous blood sample was drawn from each child before transfusion. Blood was allowed to clot and serum was separated and stored in freezer, at -80ºC, for analysis later on. Following parameters were evaluated.

Serum Thyroid Profile:

1. Triiodothyronine (T_3): For the quantitative determination of the triiodothyronine concentration in the serum, enzyme immunoassay kit was used.
Normal range:- 0.52-1.85 ng/ml

2. Thyroxine (T_4): The total thyroxine concentration was determined using T_4 enzyme immunoassay kit. Normal range:- Male: 44-108 µg/dl Female: 48-116 µg/dl

3. Serum Thyroid stimulating hormone (TSH): Thyroid stimulating hormone in the serum was determined by Elisa Microwell kit.
Normal range:- 0.39-6.16 µU/ml

4. Serum Ferritin: Serum ferritin levels were determined by using Accu-Bind Elisa Microwell kit.
Normal range:- Male: 16-220 µg/L Female: 10-124 µg/L

Statistical Analysis: The data was analyzed using SPSS version 17.0 (Statistical Package for Social Sciences). Mean ± SEM was calculated for all quantitative variables like (T_3, T_4, TSH and serum ferritin). Pearson correlation coefficient was applied to observed correlation between serum ferritin level and thyroid tests. A p-value of ≤0.05 was considered statistically significant.

RESULTS

In the study there were a total of 90 subjects, out of whom 40(44.44%) were females and 50(55.6%) were males. The male to female ratio is 1.14:1. The mean age of the patients was 12.04 ± 02.0 years.

The most frequently occurring blood group among patients was O^+, constituting 49%; 44 patients out of all 90. The second foremost found blood group was B^+ with 26.7% patients with proceeding group of A^+ comprising 16% of patients. Only 7% patients comprised of all remaining blood groups i.e. A^-, AB^+, B^-, and O^-.

The mean value of Triiodothyronine taken as a whole was 1.146±0.02, with a minimum of 0.30 and a maximum of 1.9. For males the mean value of Triiodothyronine was 1.124±0.04 and for females it was 1.175±0.03. The minimum and maximum values for males were 0.30 and 1.9 and for males 0.8 and 1.5 respectively. There was a significant mean difference of Triiodothyronine observation among males and females (p-value=0.043). Table 1.

Table No.1: Descriptive Statistics of Study Parameters

Parameters\	Mean ± SE		Minimum Value		Maximum Value		p-value
	Male	Female	Male	Female	Male	Female	
Serum Triiodothyronine (ng/ml)	1.124±0.04	1.175± 0.03	0.30	0.80	1.9	1.5	0.043
Serum Thyroxine (µg/dl)	90.24± 41.1	96.59± 32	39	71	142.3	136.5	0.243
Thyroid Stimulating Hormone (µIU/ml)	3.67± 0.69	4.73± 1.20	0.60	0.8	34.6	42.0	0.143
Serum Ferritin (ng/ml)	4601.6±187.5	4996.28±184.60	716.3	2360.0	7616	7070	0.143

For thyroxine, the mean value for all subjects was found to be 93.06±27.0 µg/ml with a minimum of 39.0 and a maximum of 142.30. The average value among males was 90.24±41.1 µg/ml with a minimum of 39.0 and maximum of 142.3. For females, the mean value of thyroxine was 96.59±32.0 µg/ml with a minimum of 71.0 and a maximum of 136.5. There was no significant difference in mean values of thyroxine between both genders (p-value=0.243). (Table-1)

Mean TSH value for all subjects was 4.14±0.65 µU/ml, with a minimum of 0.60 and maximum of 42.00. The minimum value of TSH in males was 0.60 and maximum value was 34.60, whereas among females these values were 0.80 and 42.00 respectively. The mean TSH values were same among both males (3.67±0.69) and females (4.73±1.20) statistically (p-value= 0.424). (Table-1)

The mean±SEM value for serum ferritin for all patients was 4777.04± 133.54 µg/L with a minimum of 716.33 and maximum of 7616.00. The minimum and maximum values among males were 716.33 and 7616.00 µg/L while among females 2360.00 and 7070.00 µg/L respectively. The mean values of serum ferritin were same among males (4601.65± 187.58) and females (4996.28± 184.60) statistically (p-value= 0.143). (Table-1).

According to the severity of ferritin level 85 (94.4%) patients had sever grade while moderate and mild ferritin level was seen in 4(4.4%) and 1(1.1%) patients respectively.

KEY:- Mild = Upto 1000 µg/L Moderate = 1000-2500 µg/L Severe: = More than 2500 µg/L

There was a weak negative correlation of Serum Ferritin with both Triiodothyronine and Thyroxine (Pearson correlation; -0.072 & -0.094 respectively). Both correlation i.e. Serum Ferritin with Triiodothyronine and Thyroxine were statistically insignificant (p-value= 0.294 & 0.189 respectively). **(Figure 1 and 2)**

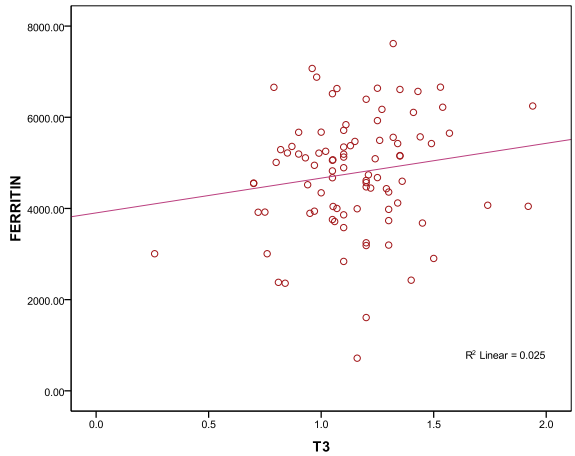

Figure No.1: Scatter plot demonstrating weak negative insignificant correlation in Serum Ferritin and Triiodothyronine

- Pearson correlation = 0.159
- p-value = 0.135 (insignificant correlation)

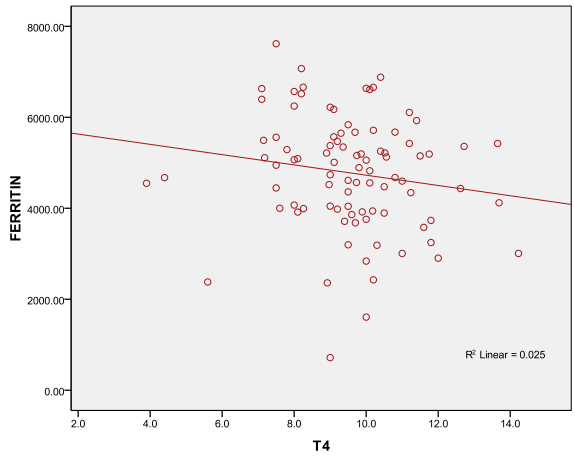

Fig-II: Scatter plot demonstrating weak negative insignificant correlation in Serum Ferritin and Thyroxine

- Pearson correlation = -0.028 p-value = 0.794 (insignificant correlation)
- Serum Ferritin had a weak positive, insignificant correlation with TSH

(Pearson Correlation 0.104; p-value= 0.164), **(Figure 3)**

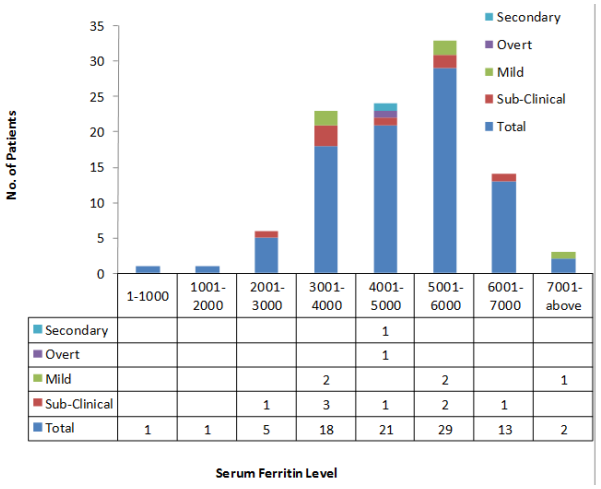

Figure No.4: Comparison of different types of hypothyroidism with serum ferritin level

DISCUSSION

Thalassemia syndromes are the result of a large number of molecular defects that alter the expression of one or more of the globin genes. More than 200 point mutations and rarely deletions have, so far, been described in β-globin gene on chromosome 11, resulting in β-thalassemia[14]. It is common in Mediterranean countries, the Middle East, Central Asia, Indian Subcontinent, Southern China, South East Asia, and Far East [15.16.17].

Programmed repeated blood transfusions, with chelation of iron are the logical therapy for thalassemia patients and have dramatically improved the quality of life and changed it, which was once a fetal disease in early childhood to chronic disease compatible with prolonged life[18]. Now a days life expectancy may extend up to late fifties in some developed countries[15]. These patients have a dramatic increase in iron absorption from the gut which is mediated by down regulation of hepcidin receptors[19.20]. The capacity of serum transferrin, the transport protein to bind and detoxify iron, are exceeded and surplus iron can lead to generation of free radicals from reactive oxygen species via Fenton reaction[21], which can cause tissue damage[22]. ROS include a wide variety of oxygen-, carbon-, and nitrogen- radicals originating from superoxide radicals, hydrogen peroxide and lipid peroxidases[23]. Hydroxy radicals facilitated by membrane associated iron might be particularly harmful because radicals' generation would be relatively sequestered from the cell's antioxidant capacity and occurs directly adjacent to membrane components[24].

Endocrine dysfunction is a frequent complication in thalassemic patients who are on regular transfusions[25]. In some reports, upto 66% patients have at least a single endocrine disorder and 40% have multiple endocrinopathies[26]. Reduced nicotinamide adenine dinucleotide phosphate-induced lipid peroxidation,

cytochrome P-450 inactivation and free radicals production are thought to be the etiology of iron toxicity[27].

Thyroid hormones, thyroxine and triiodothyronine have potent effects on growth, development, and metabolism in almost all tissues of the body, after binding to thyroid hormone receptors [28.29]. Thyroid dysfunction has been reported ranging from a low prevalence of 0-12% to a high prevalence of 10-35% in different cohorts[10]. Hypothyroidism is the second major and well-documented endocrine complication resulting from hemosiderosis[11]. After about one year of transfusions, iron begins to accumulate in parenchymal tissues where it may cause substantial toxicity as compared to that in reticuloendothelial tissues[12].

Results in this study showed that 88 patients (97.8%) had serum ferritin level higher than 2000 μg/L which is very high as compared to levels reported from some developed countries. In these centres only 19 (21.11%) patients got their ferritin levels checked once a year. Hypothyroidism was discovered in 15 patients (16.67%). Out of these 8 patients (8.89%) were having subclinical hypothyroidism with normal serum thyroxine level and slight raised TSH level (≤10 μU/ml). Ferritin level in all these levels was more than 2000 μg/L. Five patients (5.56%) were suffering from mild hypothyroidism with normal T_4 and high TSH levels (more than 10 μU/ml). One patient (1.11%) was diagnosed as having overt hypothyroidism with low T_4 and TSH levels. Ferritin level in this patient was 4548.33 μg/L. Hypothyroidism was not detected in whom serum ferritin level was below 2000 μg/L, indicating better control of iron overload and resultantly no thyroid damage.

No significant correlation between ferritin and T_3 and T_4 levels was reported by some other researchers[15.32.].This may be, in part, due to the fact that ferritin levels increase linearly with transfusion overload up to about 100 units of transfused blood, but afterward, there is no simple relationship. Another hypothesis suggested by Pirinccioglu et al is that damage of endocrine glands caused by chronic hypoxia is more prominent than that caused by hemosiderosis.

A high prevalence of hypothyroidism, in patients under study, may be due to ignorance about chelation therapy, its cost and regularity, and carelessness of the parents. Moreover availability of chelating agents and its standardization are some other problems encountered in these centres. Symptoms of hypothyroidism might be confused with that of anemia by clinicians, so at times hypothyroidism might be overlooked especially in adolescent age.

CONCLUSION

In the absence of any significant correlation, it is essential that ferritin level should not be relied upon solely while investigating organ damage in these patients. The role of thyroid hormone in the growth and development in children and availability of hormone replacement therapy necessitates frequent estimation of thyroid status to avert tissue damage and consequent complications.

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