Correlation Between Serum Ferritin and Gonadotrophins and Sex Hormones in Patients with Transfusion Dependent β-Thalassemia

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ABSTRACT

Background: Patients with transfusion dependent beta thalassemia major develop a number of complications of which hypogonadism is the most common. In addition, raised serum ferritin level has been associated with hypogonadism.

Objective: To determine a correlation between serum ferritin, gonadotrophins and sex hormones in patients with transfusion dependent β-thalassemia syndrome patients.

Material and methods: This cross sectional study was designed and conducted at the Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU) Peshawar and Fatimid Foundation, blood bank and hematological services Peshawar. Beta thalassemia syndrome patients enrolled in the study were 97. Blood samples collected from the study subjects were analyzed at IBMS KMU using chemiluminescence immune assay for determination of serum ferritin and gonadal hormones such as FSH, LH, Estradiol and Testosterone.

Results: The mean±SD age of patients was 18.93±3.6 years with a range of 15-32 years. There were 55 (56.7%) males and 42 (43.3%) females. Overall hypogonadism was diagnosed in 39 (40.21%) patients. Although we did not find any association between serum ferritin level and gonadal function in our study, hypogonadism having males had a higher serum ferritin level (p = 0.02). A statistically significant negative correlation was found between serum ferritin and serum level of LH, FSH and testosterone in males (p <0.05).

Conclusion: The effect of iron overload on serum level of gonadotrophins and gonadal hormones is more pronounced in males as compared to female thalassemic patients.

Keywords: Beta Thalassemia syndrome, Chelation, Ferritin, Hypogonadism, Gonads, Sex Hormones.

INTRODUCTION

The beta thalassemia syndrome is a constellation of a number of diseases characterized by a deficient or no synthesis of normal globin chains leading to a skewed synthesis of beta chains of hemoglobin.¹ This imbalance in the globin chain synthesis results in ineffective production of erythrocytes as well as hemolysis causing anemia in these patients. ² Categorized as thalassemia minor and thalassemia major, the latter form of beta thalassemia is characterized by anemia which manifests a life-long dependence on transfusion of blood or blood products.³

However, the dependence of thalassemic patients on blood transfusions almost always results in secondary iron-overload which, when left untreated, has been known to cause organ-specific toxicity as well as death.⁴ Four out of five children born each year with an inherited disorder of globin synthesis such as thalassemia or sickle cell disease are born in a tropical belt of low to middle income countries that stretches from Southeast Asia in the east through to the Middle East to the Mediterranean and the Sub-Saharan Africa.⁴

Hypogonadism is one of the many endocrine complications associated with thalassemia major. It is known to be present in as many as 80% of patients with thalassemia.⁵ ² Hypogonadism in thalassemia is known to be associated with a number of secondary complications such as infertility, sexual dysfunction, delayed puberty, short stature, osteoporosis and disproportionate body growth.⁵-⁷ The endocrinopathies of beta thalassemia syndromes is frequently attributed to chronicity of anemia as well as toxicity of iron accumulated in tissues particularly in pituitary glands as a result of long-term blood transfusion.⁸

In addition to older age, lower hemoglobin level and high serum ferritin levels⁵, other risk factors that have been found to be associated with an increased occurrence of hypogonadism in patients with thalassemia major include beginning blood transfusions at an early stage of the disease, poor compliance with the chelation therapy.⁵, ¹³ A number of studies have sought to explore the association, if any, between serum ferritin levels and sex hormones such as follicle stimulating hormone (FSH), Luteinizing Hormone (LH), Estrogen and testosterone and varying results have been reported.¹⁴-¹⁷
Keeping in view the variable association of gonadal hormones with serum ferritin, this study was designed to determine a correlation between serum ferritin level and serum levels of other hormones such as FSH, LH, estradiol, and testosterone. Determination of such correlation could prove valuable since serum ferritin is also an important prognostic factor in conditions characterized by iron overload. Hence, determination of serum ferritin could be used to identify patients at risk of developing endocrinopathies in general and hypogonadism in particular.

MATERIAL AND METHODS

This cross-sectional study was designed and conducted at the Institute of Basic Medical Sciences (IBMS), Khyber Medical University (KMU) Peshawar and Fatimid Foundation, Blood Bank and Haematological Services Hayatabad Peshawar wherein blood samples were collected from patients with beta thalassemia at the Fatimid Foundation, blood bank and haematological services Hayatabad Peshawar. Samples were analyzed at the pathology lab of IBMS KMU Peshawar. The study duration was from March 1, 2014 to March 31, 2015. The study participants were enrolled after obtaining permission from relevant authorities as well as following explanation of purpose and objectives of the research to patients themselves or guardians of the thalassemic patients. The study sample size consisted of 97 thalassemic patients. This number was calculated using the following formula for sample size calculation:

\[ n = \frac{Z^2 P (1-P)}{e^2} \]

Where Z (Level of significance) = 1.96
P (Prevalence) = 14.29%
e (margin of error) = 7%

A non-probability consecutive sampling technique was used to include thalassemic patients of either gender aged 15 yrs or above who had been transfused in multiple for thalassemia major. Patients who did not consent for participation in the study, hormone-replacement therapy recipients and patients with comorbidities such as diabetes mellitus, hypertension or coronary artery disease were excluded from the study as inclusion of such patients could introduce bias into the study results. For the purpose of this study, multiple transfusions were defined as at least twelve blood transfusions in a thalassemic patient. Following enrollment into the study, a comprehensive medical history of each patient was recorded including their contact telephone number, their blood group, age at which thalassemia was diagnosed, age at which the patients received their first blood transfusion, the frequency with which they received blood transfusions, gap of time between two consecutive blood transfusions, if and when they received chelation therapy, its type and frequency with which they received it, menstrual history in case of females and presence of co-morbid conditions. Each study participant was thoroughly examined and a record of their pulse rate, presence of pallor, their height and weight, splenomegaly and secondary sexual characteristics of patients such as facial hair in boys and breast development in girls was made. Blood samples were collected from each patient under strict aseptic conditions from the ante-cubital veins in arms.

The blood was analyzed using chemiluminescence Immune assay for determination of serum ferritin (by Immunoenzymometric Sequential Assay type 4) and gonadal hormones such as FSH, LH (by Immunoenzymometric assay Type 3), Estradiol (by delayed Competitive enzyme immunoassay type 9) and Testosterone (by Competitive Enzyme Immunoassay Type 7). The reagents and kits used were of Monobind Incorporation USA.

The study sample was further grouped into pediatric and adult groups at the time of data analysis with a cut-off age of 16 years. Study participants younger than 16 years were grouped into pediatric age group and those older than 16 years were grouped as adults.

The data collected was entered into and analyzed using SPSS v 20. Numerical data was described as mean and standard error. Student's t-test and Pearson correlation coefficient were used to determine significance of relationship between serum ferritin and different gonadal hormones.

RESULTS

The mean age of patients was 18.93±3.6 years with a range of 15-32 years. Total number of male participants were 55 (56.7%) while females were 42 (43.3%) in this study. A significant majority n=69 of the study was younger than 16 years of age. Most of study population 71.13% had delayed puberty as evident from the fact that among males 83.65% did not have any facial hair development while 54.7% females did not have any breast development even at the age of 15.
The mean serum ferritin level in the study population was 12848±4954.6 ng/ml with a range of 1500-27042 ng/ml. 31 out of 55 males (56.4%) and 8 out of 42 (19.05%) females were found to have hypogonadism in our study and overall hypogonadism was diagnosed in 39 (40.21%) patients with transfusion dependent beta thalassemia.

Hypogonadism was diagnosed on the basis of serum testosterone levels in males and serum estradiol levels in females. The following cut-off levels of both hormones for diagnosis of hypogonadism were used: Serum testosterone levels less than 0.028 ng/ml for thalassemic patients younger than 16 years of age and 2.5 ng/ml for thalassemic male patients aged 16 and above. On the other hand, the serum estradiol cut-off level for females was 15 pg/ml irrespective of their age. The gonadal functions of male and female study participants in both pediatric and adult groups is shown in tables-1 and 2 below.

**Table 1** Hormonal analysis of gonadal functions in males

| Variables          | Whole Sample Mean ± SD | Eugonadism Mean ± SD | Hypogonadism Mean ± SD |
|--------------------|-------------------------|-----------------------|-------------------------|
| **Parameters**     | N = 55                  | Paediatrics group N= 17 | Paediatrics group N= 17 |
| S. Testosterone (ng/ml) | 1.94 ± 2.97             | .05 ± .022            | .05 ± .01               |
| S. FSH (mIU/ml)    | 5.10 ± 5.21             | 3.57 ± 3.99           | 3.70 ± 3.26             |
| S. LH (mIU/ml)     | 2.05 ± 2.59             | .56 ± .65             | .90 ± .76               |
| **Parameters**     | N = 55                  | Adult group N= 32     | Adult group N= 32       |
| S. Testosterone (ng/ml) | 1.94 ± 2.97             | 5.94 ± 2.62           | .48 ± .75               |
| S. FSH (mIU/ml)    | 5.10 ± 5.21             | 11.11 ± 2.81          | 1.87 ± 3.83             |
| S. LH (mIU/ml)     | 2.05 ± 2.59             | 5.02 ± 3.00           | .90 ± 1.00              |

SD, Standard Deviation; n, Number of patients; S., Serum; FSH, Follicle Stimulating Hormone; LH, Luteinizing Hormone; ng/ml, nanograms per millilitre; mIU/ml, Milli international units per millilitre

**Table 2** Hormonal analysis of gonadal functions in females

| Variables          | Whole Sample Mean ± SD | Eugonadism Mean ± SD | Hypogonadism Mean ± SD |
|--------------------|-------------------------|-----------------------|-------------------------|
| **Parameters**     | N= 42                   | Paediatric group N= 11 | Paediatric group N= 11 |
| S. Estradiol (pg/ml) | 70.57 ± 57.23           | 68.10 ± 38.21         | 7.66 ± 3.04             |
| S. FSH (mIU/ml)    | 10.73 ± 6.79            | 9.52 ± 5.17           | 3.03 ± 4.21             |
| S. LH (mIU/ml)     | 2.89 ± 2.50             | 2.48 ± 2.97           | 1.26 ± .94              |
| **Parameters**     | N= 42                   | Adult group N= 31     | Adult group N=31        |
| S. Estradiol (pg/ml) | 70.57 ± 57.23           | 90.60 ± 57.35         | 8.16 ± 2.05             |
| S. FSH (mIU/ml)    | 10.73 ± 6.79            | 13.18 ± 6.35          | 4.50 ± 5.47             |
| S. LH (mIU/ml)     | 2.89 ± 2.50             | 3.33 ± 2.63           | 2.22 ± .92              |

SD, Standard Deviation; N, Number of patients; S., Serum; FSH, Follicle Stimulating Hormone; LH, Luteinizing Hormone; pg/ml, Picograms per millilitre; mIU/ml, Milli international units per millilitre

Pediatric group = age less than 16 years  Adult group = age more than 16 years.
We did not find any association between serum ferritin levels and the gonadal function in our study participants. Interestingly, the mean serum ferritin level was significantly higher in male patients with hypogonadism (Table-3) while no such effect was observed with the female study participants (Table-4).

**Table 3** Comparison of means of serum ferritin between eugonadal and hypogonadal groups of males

| Variables | Testosterone based groups | N   | Mean       | Std. Deviation | P value |
|-----------|---------------------------|-----|------------|----------------|---------|
| Current S. Ferritin (ng/ml) | Eugonadal                | 24  | 10947.63   | 4799.76        | .02     |
|           | Hypogonadal               | 31  | 14297.26   | 5576.53        |         |

N, Number of patients; Std, standard; S., serum; ng/ml, nanograms per millilitre

**Table 4** Comparison of means of serum ferritin between eugonadal and hypogonadal groups of females

| Variables | Testosterone in adult group | N   | Mean       | Std. Deviation | P value |
|-----------|-----------------------------|-----|------------|----------------|---------|
| Current S. Ferritin (ng/ml) | Eugonadal                | 34  | 13005.21   | 4395.92        | .667    |
|           | Hypogonadal               | 8   | 12272.50   | 3795.64        |         |

N, Number of patients; Std, standard; S., serum; ng/ml, nanograms per millilitre

Among both male and female study participants, no statistically significant association was found between serum ferritin and gonadal function ($p > 0.05$). (Table-5 and Table-6).

**Table 5** Cross table between iron overload and gonadal functions in males

| Ferritin grouping | Gonadal groups | Total | Value | P   |
|-------------------|----------------|-------|-------|-----|
|                   | Eugonad | Hypogonad |       |     |
| Moderate iron overload |      |           | 3     | 1   | 4   |
| Count % of Total  | 5.5%    | 1.8%       | 7.3%  | .23 |
| Severe iron overload |      |           | 7     | 6   | 13  |
| Count % of Total  | 12.7%   | 10.9%      | 23.6% |     |
| Very severe iron overload |      |           | 14    | 24  | 38  |
| Count % of Total  | 25.5%   | 43.6%      | 69.1% |     |
| Total             | 24     | 31         | 55    |     |
| Count % of Total  | 43.6%   | 56.4%      | 100.0%|     |

**Table 6** Cross table between iron overload and gonadal functions in females

| Ferritin grouping | Gonadal groups | Total | Value | P   |
|-------------------|----------------|-------|-------|-----|
|                   | Eugonad | Hypogonad |       |     |
| Moderate iron overload |      |           | 1     | 0   | 1   |
| Count % of Total  | 2.4%    | 0.0%       | 2.4%  | .605|
| Severe iron overload |      |           | 8     | 2   | 10  |
| Count % of Total  | 19.0%   | 4.8%       | 23.8% | 1.000|
| Very severe iron overload |      |           | 25    | 6   | 31  |
| Count % of Total  | 59.5%   | 14.3%      | 73.8% |     |
| Total             | 34     | 8          | 42    |     |
| Count % of Total  | 81.0%   | 19.0%      | 100%  |     |
When correlations between serum ferritin and the serum levels of gonadal hormones were analyzed in both males and females, different patterns were observed. For example, in male study participants, serum ferritin was found to be negatively correlated with the serum LH \((p = .019)\), FSH \((p = .001)\) and serum testosterone \((p = .049)\) and this negative correlation was statistically significant suggesting that increase in serum ferritin level negatively affects the serum levels of gonadal hormones in male study participants. The male gonadal hormones i.e., serum LH, FSH and serum testosterone were found to have strong, statistically significant positive inter-relations among themselves \((p = .001)\) (Table-7).

Among female study participants, although similar strong positive statistically significant inter-relations were observed among gonadal hormones \((p < .05)\), no statistically significant correlation was found between serum ferritin and serum levels of LH, FSH and Estradiol (Table-8).

**Table 7** Pearson Correlation between serum Ferritin and gonadal functions in males

|                | Current S. Ferritin (ng/ml) | S. FSH (mIU/ml) | S. LH (mIU/ml) | S. Testosterone (ng/ml) |
|----------------|-----------------------------|-----------------|----------------|-------------------------|
| S. Ferritin    | Pearson Correlation         | 1               | -.519\**      | -.315\*                  | -.266\*                  |
| Sig. (2-tailed)| N                           | 55              | 55             | 55                      | 55                       |
| S. FSH (mIU/ml)| Pearson Correlation         | -.519\**        | 1              | .515\**                  | .634\**                  |
| Sig. (2-tailed)| N                           | 55              | 55             | 55                      | 55                       |
| S. LH (mIU/ml)| Pearson Correlation         | -.315\*         | .515\**        | 1                       | .867\**                  |
| Sig. (2-tailed)| N                           | 55              | 55             | 55                      | 55                       |
| S. Testosterone (ng/ml) | Pearson Correlation         | -.266\*         | .634\**        | .867\**                 | 1                        |
| Sig. (2-tailed)| N                           | 55              | 55             | 55                      | 55                       |

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed)
S., Serum; N, number of patients; FSH, Follicle stimulating hormone; LH, Luteinizing hormone; ng/ml, nanograms per milliliter; mIU/ml, milli international units per millilitre

**Table 8** Pearson Correlation between serum Ferritin and gonadal functions in females

|                | Current S. Ferritin (ng/ml) | S. FSH (mIU/ml) | S. LH (mIU/ml) | Serum Estradiol (pg/ml) |
|----------------|-----------------------------|-----------------|----------------|-------------------------|
| S. Ferritin    | Pearson Correlation         | 1               | .092           | .044                    | -.003                   |
| Sig. (2-tailed)| N                           | 42              | 42             | 42                      | 42                       |
| S. FSH (mIU/ml)| Pearson Correlation         | .092            | 1              | .468\**                 | .494\**                 |
| Sig. (2-tailed)| N                           | 42              | 42             | 42                      | 42                       |
| S. LH (mIU/ml)| Pearson Correlation         | .564            | .002           | .001                    | .004                    |
| Sig. (2-tailed)| N                           | 42              | 42             | 42                      | 42                       |
| Serum Estradiol (Pg/ml) | Pearson Correlation         | .784            | .468\**        | 1                       | .430\**                 |
| Sig. (2-tailed)| N                           | 42              | 42             | 42                      | 42                       |

** Correlation is significant at the 0.01 level (2-tailed)
S., Serum; N, number of patients; FSH, Follicle stimulating hormone; LH, Luteinizing hormone; pg/ml, picograms per milliliter; mIU/ml, milli international units per millilitre
DISCUSSION

Endocrine disorders are frequently seen in transfusion dependent thalassemic patients. The situation is further aggravated by inadequate iron chelation therapy which leads to deposition of iron and its free radicals in the hypothalamus-pituitary-gonads axis. Delayed puberty and hypogonadotropic hypogonadism are the most common endocrinopathies seen as a result of this deposition of iron.

Iron deposition is not just limited to the hypothalamic-pituitary-gonadal axis organs, it is seen in other organs such as the heart and the liver. The high prevalence of hypogonadotropic hypogonadism in these patients reflects the loss of gonadal function as well as the absence of sexual maturation in these patients, despite the fact that the exact mechanism by which iron deposition causes hypogonadotropic hypogonadism is not clearly known.

It is uncommon to see thalassemic males becoming fathers to their offspring. On the other hand, transfusion dependent thalassemia females have gone to become mothers. In view of increased life expectancy of thalassemic patients as a result of interventions such as iron chelating therapy and blood transfusions, preservation and evaluation of the reproductive function of male patients becomes important than ever.

The role of iron toxicity in impairing the reproductive function of thalassemic patients is well known. In our study 39 (40.21%) patients had hypogonadism and these included 31 males and 8 females. A negative correlation was found between serum ferritin level and male gonadotropin function and it was found to be statistically significant corroborating earlier reports, while no statistically significant correlation was noted between these in the female thalassemic patients. This observation is interesting, since Sharaf et al reported that no statistically significant relationship exists between serum ferritin and serum levels of gonadal hormones such as the LH, FSH, estradiol and/or testosterone. However, their study sample was small compared to our study sample, i.e., 30 vs 97. The mean serum ferritin level in their study was also much lower (3328.94±2195.73 ng/ml) than observed in our study having level of 12848±4954.6 ng/ml.

A study that aimed to evaluate the functional status of hypothalamic-pituitary-gonadal axis in male adolescents diagnosed with β-thalassemia major, found that there was a very significant correlation of serum ferritin with hypogonadotropic hypogonadism, similar to our results. However, in addition to finding the difference in mean serum ferritin levels between the eugonadism and hypogonadism groups to be significant, we found that there was a statistically significant negative correlation between serum ferritin and individual gonadotropins in male study participants only.

Though Al-Rimawi and colleagues did report that the serum ferritin levels were high in their study participants with hypogonadotropic hypogonadism, they did not determine a correlation between serum ferritin with individual hormones in their study participants. Moreover, their study sample consisted only of males with β-Thalassemia Major and they did not include female adolescents with β-Thalassemia Major.

High serum ferritin levels (more than 2500 ng/ml) in patients with β-Thalassemia Major have been found to be associated with hypogonadism.

Interestingly, a study from Bangladesh recently reported that there was no statistically significant correlation between serum ferritin level and various endocrine complications associated with β-Thalassemia Major. Similar observations have been made by Abo-Elwafa and colleagues recently. Another study which sought to determine the effect of serum ferritin level on gonadotropins and gonadal hormones as well as the correlation between the serum ferritin and gonadal hormones and gonadotropins in patients with β-Thalassemia Major found that iron overload was associated with a significant reduction of gonadotropin and gonadal hormone levels in thalassemic patients. The study also reported a significant negative correlation between serum ferritin and serum levels of FSH, LH, testosterone and estradiol (p < 0.05). This correlation was seen in both male and female thalassemic patients.

While these results are similar to our findings, we found a positive statistically significant interrelationship between gonadal hormones and gonadotropins in both males and females, and we didn't observe statistically significant correlation between serum ferritin and the gonadal hormones in the female thalassemic patients.

The endocrinopathies associated with β-Thalassemia Major are related to the deposition of
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iron in different organs of body and hypogonadism being the most common. We have observed that the effect of iron overload on the serum level of gonadotrophins and gonadal hormones is more pronounced in male thalassemia patients compared to the female thalassemic patients. One of the reason is that males are more privileged in our society. They need not be accompanied, can manage alone and usually receive more blood transfusions than females with a consequence of having more iron deposition in all the organs and their malfunction. But still to determine the true nature of this predilection of male sex with iron-overload associated hypogonadism, future studies with a larger sample size are required.

CONCLUSION

The effect of iron overload on serum level of gonadotropins and gonadal hormones is more pronounced in males as compared to female thalassemia patients.

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AUTHOR’S CONTRIBUTION
Following authors have made substantial contributions to the manuscript as under

Khan H, Ilyas: Concept and design of study, Collection of data, statistical analysis

Orakzai SA, Alam S: Writing of manuscript, critical review of manuscript

Alam S, Roghani: Analysis and interpretation of data, statistical analysis

Naveed MM, Ullah U: Data collection, bibliography

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.