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Effect of Iron Overload on Gonadotrophins and Organ Sex Steroids in Pubertal Thalassemia Patients

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ABSTRACT

Background: Iron overload being one of the major adverse effect related to blood transfusion in β Thalassemia major,leads to various endocrinal disorders among which hypogonadism is noteworthy.Pituitary or gonadal iron deposition or both may lead to this.

Objectives: The main purpose of this study was to evaluate the effect of iron overload measured by serum ferritin on pituitary gonadotrophins (FSH, LH) and gonadal steroids (Estrogen in females,Testosterone in males) levels and the correlation between them in pubertal thalassemic subjects(cases).

Methods: Serum harvested from blood samples collected from 30 cases(15 males,15 females) and 30 controls were used to estimate Serum ferritin, LH, FSH, Estradiol in females and Testosterone in males.

Results: The serum LH (p<0.001), FSH (p<0.001), Estradiol in females (p 0.002) and Testosterone in males (p 0.002) were significantly low in cases than controls. There was significant negative correlation between Ferritin:FSH (ρ-0.706, P<0.001-females, ρ-0.838, P<0.001-males), Ferritin: LH (ρ-0.885, P<0.001-females, ρ-0.806, P<0.001-males) Ferritin: Estradiol (ρ-0.584, P<0.001-females), Ferritin: Testosterone (ρ-0.664, P<0.001-males) and significant positive correlation between FSH:LH (ρ0.623, P<0.001-females, ρ-0.871, P<0.001-males), FSH: Estradiol (ρ0.839, P-0.01- females), FSH: Testosterone (ρ-0.860, P-0.001-males), LH: Estradiol (ρ0.913, P<0.001-females, LH: Testosterone (ρ-0.849, P<0.001-males).

Conclusions: The current study reveals that iron overload has significantly reduced pituitary gonadotrophins as well as gonadal sex steroids,but the effect being more due to iron deposition in pituitary as indicated by the higher rho(ρ) value between ferritin and LH, FSH than between ferritin and estradiol, testosterone. The deficiencies of gonadotrophins have failed to stimulate the gonads leading to hypogonadotrophic hypogonadism and delayed puberty.

Key Words: β Thalassemia major, Gonadotrophins, Hypogonadism, Delayed puberty

INTRODUCTION

β-thalassemia is the commonest single-gene disorder in the Indian population [1]. Ten percent of the total world thalassemics 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]

Iron overload which is a common complication of thalassaemic syndromes could lead per se to the development of organ damage and increased mortality [4]. In these patients, iron deposition in parenchymal tissues starts within 1 year of starting the regular transfusions [5]. Blood transfusions are important for survival of these patients, but chronic transfusions inevitably lead to iron overload as humans cannot remove excess iron actively. The cumulative effects of iron

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overload, if untreated, lead to significant morbidity and mortality [6]. The current management of thalassemia (TM) includes regular transfusion programs and chelation therapy. Current guidelines recommend a pretransfusion threshold not exceeding 9.5% g/dl, which seems to be associated with adequate marrow inhibition and a relatively low iron burden. [7] Research also indicates that iron overload can occur in patients with non-transfusion dependent thalassaemias(NTDT) [8]

Delayed puberty and hypogonadism are among the most common clinical consequences of iron overload. Iron deposition in the pituitary gonadotrophic cells leads to disruption of gonadotropin (LH and FSH) production. In the majority of well-chelated patients, the gonadal function is normal; however, gonadal iron deposition occasionally occurs. TM patients with a favorable genotype manifest less severe gonadal dysfunction, due to less iron loading. [9] The precise mechanism of iron overload-induced organ dysfunction leading to delayed puberty is not clear. It is still not known whether iron deposition in the pituitary gonadotrophic cells or gondal iron deposition or both causes the hypogonadism. Some studies[10] found significant difference in mean serum ferritin level between thalassemic patients with primary amenorrhea, irregular mense, hypogonadism and those without endocrinopathies. These findings yield the importance of iron overload in development of endocrine disorders among which hypogonadism is most frequent. In contrast, there are some othereports which have suggested no relation between the level of ferritin and some other endocrinopathies [11,12]. Based on the above mentioned information, we set out to study the effect of iron overload on pituitary gonadotrophins and the gonadal sex steroids in pubertal thalassemics and to conclude whether the former or later is most affected.

AIMS AND OBJECTIVES:

1. To measure serum ferritin to detect iron overload pubertal thalassemic patients(cases) as well as in controls
2. To measure pituitary gonadotrophins-Luteinizing hormone (LH), Follicle Stimulating Hormone (FSH) in the study subjects (cases and controls).
3. To measure gonadal steroids-Estrogen in female subjects, Testosterone in male subjects
5. To assess any correlation between serum ferritin, pituitary gonadotrophins and gonadal steroids in the case group.

MATERIALS AND METHOD:

Study type: It was a hospital based observational study

Study design: The study design was cross-sectional and non interventional.

Study population: The study population included pubertal thalassemic patients. The control group were selected from the healthy relatives of the patients who will accompany them being age matched.

Selection criteria:

Cases were taken from the thalassemia unit of the institution as per the following criteria:

***INCLUSION CRITERIA**

a) The age of the β-thalassemia major subjects were between the ages of 13to 17yrs to detect pubertal delay
b) They had already received multiple blood transfusions or iron therapy.

***EXCLUSION CRITERIA**

a) The newly diagnosed patients were excluded.
b) The subject who were diabetic and had other inborn metabolic diseases.
c) Subject who had active infection and inflammation.
d) Subjects who were alcoholics and cigarette smokers.

Controls were selected from healthy individuals (without any metabolic diseases, haematological diseases, chronic or active infection and inflammation).

Place of study: Selection of cases was done from the thalassemia unit of the institution.

Biochemical investigations and result analysis were performed in the dept. of Biochemistry,

Calcutta National Medical College, Kolkata.

Study duration: Two months after getting approval for the project as well as the instituitional ethical clearance certificate.

Sample size: Following inclusion and exclusion criteria thirty patients- fifteen male and fifteen female were selected by the method of convenience in each group of the cases and controls.

Ethical considerations: The study was conducted by strictly adhering to the guidelines from the Helsinki declaration, 1975 revised in 2000. Written and informed consents were obtained from participants as per protocol.

Data collection procedure: The valid written consents of the subjects were taken and proper ethical guidelines were followed. The patients were asked to come at a specific date. We collected demographic and anthropometric data and the history of menstruation, family history of diabetes, initiation and duration of blood transfusion, as well as chelation therapy. After the collection of the samples and carrying them to the Department of Biochemistry, the clotted blood was centrifuged at 1500 rpm for 5 min and the serum was harvested from which estimation of serum ferritin, Luteinizing hormone(LH), Follicle Stimulating hormone (FSH), Estra-

diol in female subjects, Testosterone in male subjects were done by ELISA.

Instruments required were:

1. ELISA Kit, ELISA Reader and Washer.
2. Micropipettes and microtips.
3. Syringes and cottons.

Principle of measurement:

- Serum ferritin- Solid phase sandwitch assay method (CalBiotech Inc.)
- Luteinizing hormone (LH), Follicle stimulating hormone(FSH)-Solid phase sandwitch ELISA (Accu-Diag)
- Estrogen, Testosterone-Solid phase competitive ELISA (DiaMetra-Italy)

In each analysis, standard and controls were tested along with the samples.

Confidentiality: Data would be kept strictly confidential and will be stored at least for three years.

Statistical analysis

The data obtained from the above tests were analyzed for differences between the medians of the analytes studied between cases (pubertal thalassemics) and controls (neonates delivered by ND).

Kolmogorov and Smirnov method

The Kolmogorov and Smirnov method was used to check whether the data were normally distributed.

Mann–Whitney U-test

In order to study the significance in the differences between the two groups, the Mann–Whitney U-test was performed for all analytes, since the data did not pass the normality test.

Spearman's correlation

Spearman's correlations were done between all analytes. All of the tests were completed with a P <0.05 as the significance level, and all statistical analyses were carried out using the SPSS 17 soft ware (SPSS Inc. Chicago, USA).

RESULTS

Table no. 1 shows that in thalassemic males (cases), there is significant iron overload as the ferritin level is significantly high (p value <0.001) compared to the control group.Pituitary gonadotrophins FSH(Follicle stimulating hormone) and LH(Lutenizing hormone) are significantly low in the case group (FSH- p value <0.001, LH- p value <0.001).There is significant difference in Testosterone level between the case and the control group (p-0.002)-the median being significantly low in the thalassemic males (cases). These results indicate gonadotrophin deficiency as well as gonadal steroid deficiency in the male Thalassemics who are sufferring from iron overload.

In thalassemic females (cases), there is significant iron overload as the ferritin level is significantly high(p value <0.001) compared to the control group as shown in Table no.2.Pituitary gonadotrophins FSH and LH levels are significantly low in the case group(FSH- p value <0.001,LH- p value <0.001).There is significant difference in 17ßEstradiol level between the case and the control group(p-0.003)-the median being significantly low in the thalassemic females(cases) These results indicate gonadotrophin deficiency as well as gonadal steroid deficiency in the female Thalassemics who are sufferring from iron overload.

Table no. 3 shows the Spearman's correlation between analytes in male cases where there is significant negative correlation between Ferritin: FSH(ρ-0.838, P<0.001), Ferritin:LH (ρ-0.806, P<0.001) and Feritin: Testosterone (ρ-0.664, P<0.001). Thus iron overload (high ferritin) has decreased the secretion of pituitary gonadotrophins-LH,FSH as well as gonadal sex steroid-Testosterone in male thalassemic cases, but iron overload has affected the pituitary more than the gonads as the rho value is less Feritin:Testosterone (ρ-0.664) than between ferritin and the gonadotrophins. There is significant positive correlation between FSH:LH (ρ0.871, P<0.001), FSH:Testosterone (ρ0.860, P-0.01), LH:Testosterone (ρ0.849, P<0.001). This indicates the deficiency of gonadotrophins has been unable to stimulate the gonads to produce Testosterone in male thalassemic cases.

The Spearman's correlation between analytes in female cases as in Table no. 4 shows significant negative correlation between Ferritin: FSH(ρ-0.706, P<0.001), Ferritin: LH (ρ-0.885, P<0.001) and Feritin: Estradiol(ρ-0.584, P<0.001). Thus iron overload (high ferritin) has decreased the secretion of pituitary gonadotrophins-LH, FSH as well as gonadal sex steroid- Estradiol in female thalassemic cases, but iron overload has affected the pituitary more than the gonads as the rho value is less between ferritin:testosterone (ρ-0.584) than between ferritin and the gonadotrophins.. There is significant positive correlation between FSH:LH (ρ0.623, P<0.001), FSH: Estradiol (ρ0.839, P-0.01), LH:Estradiol (ρ0.913, P<0.001). This indicates the deficiency of gonadotrophins has been unable to stimulate the gonads to produce Estradiol in female thalassemic cases. The average age of puberty onset in the thalassemic cases in current study was 15.5±2 yrs which indicates delayed puberty in the male as well as female thalassemic cases.

DISCUSSION

In normal individuals, iron absorption controls iron homeostasis mainly rather than iron excretion excretion [13]. Thalassemic patients receiving a blood transfusion (usually

1mg of iron per 1mL of blood) inevitably experience significant iron overload as they lack adequate excretory mechanisms. Normally, iron bound to transferrin is transported to bone marrow and tissue, where iron is taken up by transferrin receptor and stored as ferritin. As a consequence of iron overload in thalassemic patients, either from blood transfusion or excessive iron absorption, transferrin is fully saturated or non-transferrin-bound iron (NTBI) is found excessively in the blood. The current study shows iron overload in both thalassemic males and females as evidenced statistically significant by the high ferritin level in them compared to controls.

Non-transferrin-bound iron (NTBI) enters non-hematopoietic cells by other cellular channels in forms that can possibly damage cells via iron-mediated cellular oxidative damage[14].

Endocrine dysfunction has been reported as the most common and earliest organ toxicity seen in iron-overloaded subjects with thalassaemia[15]. A study observed disease-specific differences in endocrinopathy may be related to observations of greater cellular oxidative injury in iron overloaded thalassemia versus sickle cell disease (SCD) subjects[16]. Thalassemics are more prone to iron mediated endocrinal gland dysfunction than other hemoglobinopathies like SCD. Hypogonadism is the most frequently reported endocrine complication, affecting 70–80% of thalassemia major patients. The prevalence and severity of hypogonadism in thalassemia major varies among studies, depending on the age group studied, genotype of thalassemia[17,18], extent of transfusion, age at the beginning and type of iron chelation therapy [18,19].The anterior pituitary gland is sensitive, in a dose-dependent fashion, to the effects of iron overload from transfusions [20]. Studies of human anterior pituitary adenomas showed that gonadotropes require more iron as compared with other pituitary cell types [21]. Thus, these cells are most affected, resulting in declining synthesis of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). A study found significant difference in mean serum ferritin level between thalassemic patients with primary amenorrhea, irregular mense, hypogonadism and those without endocrinopathies [10].The current study shows statistically significant low FSH and LH levels as well as significant negative correlation between ferritin and the gonadotrophins in both male and female thalassemic group.

Hypogonadism is likely to be caused by iron deposits in the gonads, pituitary gland or both. However, hypogonadotropic hypogonadism resulting from iron deposition in the pituitary gonadotrope is more commonly found. Gonadal iron deposition in ovaries or testes occurs less frequently, as the majority of amenorrheic women can still ovulate after hormonal treatment [13]. The direct effect of iron, in particular that of NTBI, on the ovaries and testes is currently unknown. The ovarian reserve is preserved in the majority of female thalassemia patients, even in women with amenorrhea. In males, histological examination of testicular tissues from autopsies demonstrated testicular interstitial fibrosis with small, heavily pigmented, undifferentiated seminiferous tubules and an absence of Leydig cells [22]. In a case report a 26-year-old female patient was referred to clinic with a 3 year history of amenorrhea. Her medical history showed a diagnosis of β-thalassemia major since the age of one and treatment with regular blood transfusions to maintain adequate levels of haemoglobin. Even after stimulation with LH Releasing hormone, pituitary response was subnormal, consistent with hypogonadotropic hypogonadism[23]. All these studies have findings similar to the current study which has revealed that iron overload has affected the pituitary more than the gonads as as the negative correlation- rho value is less negative between Feritin and Testosterone(ρ-0.664) as well as Estradiol(ρ-0.584) than between ferritin and the gonadotrophins.(Table 3,4). The deficiency of gonadotrophins has been unable to stimulate the gonads to produce Estradiol in female thalassemic cases and Testosterone in male thalassemic cases as the current study indicated significant positive correlation between FSH: Testosterone(ρ0.839,P0.01), LH: Testosterone(ρ0.913,P<0.001), FSH: Estradiol(ρ0.839,P-0.01), LH: Estradiol (ρ0.913,P<0.001). HPG axis dysfunction can manifest as low estradiol or testosterone with low to normal serum LH and FSH as commonly seen in hypogonadotropic hypogonadism. There are three main clinical presentations of the HPG axis derangement in thalassemia major, including delayed puberty, arrested puberty and hypogonadism. Delayed puberty is defined as the absence of any pubertal signs by 14 years in boys and 13 years in girls [24]. Arrested puberty is defined as the absence of further pubertal progression for more than 1 year after puberty has started.

With modern medications, iron-induced hypogonadism may be reversible with intensive iron chelation regimens [25] .Sex steroid or pulsatile GnRH can be utilized to induce puberty if the HPG axis is functionally intact, especially at an early stage of hypogonadotropic hypogonadism. Later on, if the HPG axis is irreversibly damaged, sex steroid replacement therapy is the only option to induce puberty. Generally, it is advisable to initiate puberty with sex steroid replacement therapy by age 13 in women and age 14 in men [26]. Factors such as severity of iron overload, liver disease and growth hormone deficiency should be considered before pubertal induction[27]. Sex steroids are important for the maintenance of normal body composition, skeletal health and induction and maturation of secondary sexual characteristics. It is the most important form of replacement therapy in patients not desirous of fertility. When fertility is desired, gonadotropin therapy is necessary to induce spermatogenesis [27]. Different treatment protocols can be used. The typical gonadotro-

pin regimen combines human chorionic gonadotropin (hCG) and FSH [28, 29]. Adverse events are reduced high-density lipoprotein cholesterol, thromboembolism,osteoporosis, increased prostatic symptoms in males and increased cardiovascular risk [30]. Therefore, testosterone therapy should be accompanied by a standardized monitoring plan and general health evaluation. Timely recognition and prevention of the endocrine complications, by early and regular chelation therapy is mandatory for the improvement of the quality of life and favourable psychological outcome of thalassemic patients.

Our study had various limitations like multiple serum ferritin measurements over time would be more valuable in predicting complications of iron overload than a single one as done by us in short span of time. Moreover, hypogonadotropic hypogonadism in thalassemia is related not only to iron toxicity on gonadotrope cells but also to adipose tissue leptin [31], liver disorders, chronic hypoxia and zinc deficiency which remains unexplored by us. Larger sample size was also required to improve the statistical importance of this study which was also one of our limitations.

CONCLUSION

The current study explores the effect of iron overload on the pituitary gonadotrophin secretion whose deficiency fails to stimulate the gonadal sex steroid secretion leading to hypogonadotrophic hypogonadism and delay of puberty affecting the lives of these thalassemic subjects. Thus intensive and timely iron chelation therapy can reverse iron induced organ damage leading to hypogonadism among various endocrinopathies. Gonadotrophins and sex steroids are the main treatment modalities for induction of puberty and fertility thus ensuring normal reproductive lives for these thalassemic patients.

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Table 1: Comparison of medians of analytes between serum of male cases, n=15(pubertal thalassemic) and male controls, n=15 (pubertal non thalassemics)

Analyte Tested	Median (Case)	Median (Control)	Mann Whitney U	P
Ferritin (ng/ml)	1619.5	32	13	<0.001
FSH (mIU/ml)	1.51	17	0.00	<0.001
LH (mIU/ml)	4.19	22	2	<0.001
Testosterone (ng/ml)	0.254	3.5	38	0.002

Table 2: Comparison of medians of analytes between serum of female cases, n=15 (pubertal thalassemic) and female controls, n=15 (pubertal non thalassemics)

Analyte Tested	Median (Case)	Median (Control)	Mann Whitney U	P
Ferritin(ng/ml)	2278	23	0.00	<0.001
FSH(mIU/ml)	1.9	18	0.00	<0.001
LH(mIU/ml)	3.67	25	13	<0.001
17ßEstradiol(pg/ml)	42	115	41	0.002

Table 3: Spearman's correlation between analytes in male cases.

Analytes	Rho value	P
Ferritin:FSH	-0.838	<0.001
Ferritin:LH	-0.806	<0.001
FSH:LH	0.0871	<0.001
Ferritin:Testosterone	-0.664	0.007
FSH:Testosterone	0.860	0.010
LH:Testosterone	0.849	<0.001

Table 4: Spearman's correlation between analytes in female cases

Analytes	Rho value	P
Ferritin:FSH	-0.706	<0.001
Ferritin:LH -0.885 <0.001		
FSH:LH	0.623	<0.001
Ferritin:Estradiol	-0.584	<0.001
FSH:Estradiol	0.839	0.010
LH:Estradiol	0.913	0.007