Evaluation of puberty in relation to iron overload in multi transfused B-thalassemia patients

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Abstract: Objective; Prospective study to determine the prevalence of delayed puberty in thalassemia major patients in Sohag University Hospital. This study also aimed to determine serum gonadotropin and sex hormone levels in these patients and correlate these to their serum ferritin. Methods; This cross sectional study included 30 Thalassemia Major Patients, aged 12 to 18 years. Puberty was assessed clinically and the laboratory values of various hormone levels were stratified with their age and sexual maturity. Relation between serum ferritin level and sex hormone was assessed. Results. of 30 patients, 66.7% had not attained puberty, and 100% of the girls had primary amenorrhea. F.S.H level was 1.45 ±1.88 mIU/ml before giving gonadotropin hormone analogue and after 4 hours of giving hormone, it was 3.78 ±4.19 mIU/ml. LH level before giving gonadotropin hormone analogue was 1.91 ±4.79 mIU/ml, while after 4 hours it was 6.52 ±7.50 mIU/ml. 88.24% of male cases had low serum testosterone level. 84.62% of female cases had low serum estradiol level. Correlation between serum ferritin with F.S.H, L.H, estradiol, testosterone before and after giving G.N.R.H analogue were statistically insignificant. **Conclusions.** Hypogonadism was frequent findings. The results support the need for vigilant clinical evaluation of puberty, as well as appropriate hormonal evaluation in poly transfused thalassemic children in order to detect and treat endocrine dysfunction early. The authors also recommend aggressive and adequate chelation from early life so that permanent damage to the endocrine glands can be prevented.
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1. Introduction.

Chronic anaemia, hypoxia and iron overload are considered to be responsible for secondary endocrine dysfunction in patients with thalassaemia. (1)

Blood transfusion therapy for thalassemia should maintain hemoglobin in the normal range, in order to prevent deleterious effects of anemia. It is also important to restrict iron overload by adequate chelation to prevent organ damage due to free radical formation.(2).

Transfusion and iron-chelation therapy have prolonged and improved the quality of life in patients with this disease, the improvement being mainly due to the decrease in mortality from heart failure.(3).

Though medical therapy has prolonged the life of these children, they tend to suffer from growth and endocrine problems which are multifactoral in etiology and may not be entirely prevented by treatment. (2).

Iron chelation therapy is the only method of iron overload control in transfusion dependent patients. Despite the use of iron chelation therapy, the pituitary gland, peripheral endocrine tissues and gonad axis are susceptible to iron deposition and damage.(4).

Iron deposition on the pituitary gonadotrophic cells which is followed by disruption of gonadotrophin production is the considerable reason of hypogonadotrophic hypogonadism. Secondary hypogonadism becomes evident later in life. (5). Impaired puberty in TM patients include delayed puberty, arrested puberty and hypogonadism. Delayed puberty was defined as the absence of breast enlargement in girls and testicular enlargement in boys by the age of 13 and 14 years respectively. Arrested puberty is defined as the absence of pubertal progression for more than one year after puberty onset, where testicular volume in boys is less than 6 to 8 ml and unchanged breast size in girls. Puberty onset delay more than 2 SD beyond the mean for sex was considered as hypogonadism. (4).

2.Patients and Methods:

Patients:

Prospective study was conducted on thirty children, 17 males and 13 females. They were selected from the Haematology clinic in Sohag University Hospital. Cases were recruited during the study period from July 2013 to June 2014. **Inclusion criteria:**

Thalassemia major patients aged 12-18 years old (girls) and 13-18 years old (boys). All patients were under regular transfusion program (every 14-100 days) with the aim of maintaining pre-transfusion hemoglobin (Hb) levels above 9 g/dl. All patients received iron chelating therapy, folic acid and multivitamin supplements.

Exclusion criteria:

Pediatric patients < 12 year old were excluded from the study. Subjects were excluded if they had received hormonal replacement therapy (such as testosterone, estrogen, progesterone, human chorionic gonadotropin, or human menopausal gonadotropin) or had accompanying disease that could cause delayed puberty. No attempt was made in this study to correlate growth, puberty, and hormonal levels with the type of chelation used.

Ethical consideration:

Approval of Sohag Faculty of Medicine Research Ethical Committee will be taken and verbal consent from the parents will be obtained.

Methods:

All cases included in this work will be subjected to the following:

Complete history with special emphasis to: Complaint, its duration. Age at the time of diagnosis of thalassaemia established, the amount of blood transfusions received, and the use of chelation therapy. Complete clinical examination with special emphasis to:

1. Weight, age, and sex.
2. Developmental assessment
3. Pallor, Jaundice and general condition.
4. Chest and cardiac examination
5. Abdominal examination with special comment on organomegaly such as hepatomegaly and splenomegaly.
6. Pubertal stages were determined by both visual inspection and palpation, using the criteria and definitions described by Marshal and Tanner). It is a scale of physical development in children, adolescents and adults. The scale defines physical measurements of development based on external primary and secondary sex characteristics, such as the size of the breasts, genitalia, testicular volume and development of pubic and axillary hair. This scale was first identified by James Tanner, a British pediatrician, and thus bears his name. (**Dick Mul A Miranda Fredrik s et al, 2001).**

Investigation:

Routine laboratory investigations which include:

1) Complete blood picture.

Method: Using Electronic counter Cell Dyne

2) Reticulocytic count.

Method: By Brilliant Cresyl blue stain (supravital stain).

3)- Serum ferritin:

Method: Architect Ferritin.

Principle: The Architect Ferritin assay is a chemiluminescent micro particle immunoassay (CMIA) for the quantitative determination of ferritin in human serum and plasma.

Normal level: 21.81-274.66 ng/ml.

4)-**Hormonal assay:**

The follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone and estradiol levels were correlated with the pubertal stage of the patient. Blood samples were taken from patients at morning at least two weeks after the previous blood transfusion.

Gonadotropin releasing hormone stimulation test: Serum follicle stimulating hormone (FSH) and luteinizing hormone (LH) were measured again 4 hours after subcutaneous injection of GnRH analogue (decapeptyl injection which contains the active ingredient of Triptorelin acetate 0.1mg Prefilled syringe). Triptorelin is a synthetic form of gonaderelin. It acts on the GnRH receptors in the pituitary gland in the same way as natural gonadorelin. This test is to detect response of pituitary to gonadotropin releasing hormone.

F.S.H (follicular stimulating hormone) & L.H (luteinizing hormone):

The FSH, LH levels were correlated with the pubertal stage of the patient.

Method: using the Architect I optical system.

The reference range for follicle-stimulating hormone (FSH)

During puberty: 0.95-11.95 mIU/ml.

Classification according to response to GnRH analogue:

No response: < 0.95 mIU/ml mild increase: 0.95-5 mIU/ml moderate increase: 5- 11 high increase: >11 mIU/ml

The reference range for luteinizing hormone is as follows:

At puberty:

Males: 0.57- 12.07 mIU/ml

Females: 0.3-31.0 mIU/M

Classification according to response to GnRH analogue:

no response: <0.3 mIU/M mild increase: 0.3- 10 mIU/M moderate increase: 10- 20 mIU/M high increase: >20 mIU/M.

3. Results

In this study, the total number of patients was 30, of them 17 patients were males (56.67%), and females were13 (43.33%).

The mean age of the patients was 13.91 ± 2.14 years (table 1). As regard anthropometric measurement of studied population the mean of weight in kg 31.4 ±7.98. The mean of height was 134.23 ±15.60 in c.m. As regard h head circumference the mean was 53.07±2.80 c.m.

Table (1): Age, sex and anthropometric measurement of studied population.

Variable	Mean ±SD or frequency
Age (year)	13.91 ±2.14
Sex	F/M 13 (43.33%)/17 (56.67%)
Weight (kg)	31.4 ±7.98
Height (cm)	134.23 ±15.60
Head circumference (cm)	53.07 ±2.80

As regard medical history of studied population, the mean age of diagnosis was15.76 ±18.34 in month. The mean frequency of blood transfusion per month was 1.06 ±0.29 in month (table 2).

As regard splenectomy operation we found that the majority of cases (76.67%) has been undergone of this operation. The mean Age of spleenectomy in year was1.09±0.29. 100% of cases took complete routine vaccination. Regarding family history of thalassemia was positive in 21 of cases (70%). As regard hepatitis c infection, only about 7 cases (23.33%) had hepatitis c infection.

Table (2): Medical history of studied population.

Variables	Statistics
Age at diagnosis in month Mean ±SD	15.76 ±18.34
Frequency of blood transfusion per month Mean ±SD	1.06 ±0.29
Spleenectomy No yes	 7 (23.33%) 23 (76.67%)
Age of spleenectomy in year Mean ±SD	1.09 ±0.29
Family history No Yes	 9 (30%) 21 (70%)
Hepatitis c infection No Yes	 23 (76.67%) 7 (23.33%)

As regard tanner staging, there were 20 cases (66.67%) had not attained puberty in stage (1). 7 cases (23.33%) were belong to stage (2). Stage (3) was about 2 cases (6.67%). There is only one case in stage (4))(table 4).

Table (3): Tanner staging of studied patients.

Tanner staging	Number (percent)
1	20 (66.67%)
2	7 (23.33%)
3	2 (6.67%)
4	1 (3.33%

Our study showed that, the mean level of serum ferritin was 3328.94 ±2195.73 ng/ml.(Table 5) As regard FSH & LH levels in studied population, the mean of F.S.H level was 1.45 ±1.88 mIU/ml before giving gonadotropin hormone analogue and after 4 hours of giving hormone, it was 3.78 ±4.19 mIU/ml. F.S.H level was low in 60.00% of cases. The P value of this test was <0.0001. As regard L.H level, the mean level of it before giving gonadotropin hormone analogue was 1.91 ±4.79 mIU/ml, while after 4 hours it was 6.52 ±7.50 mIU/ml. L.H level was low in 50.00% of cases The P value of this test was <0.0001.

As regard testosterone level in males, the mean level of testosterone was17 ±0.68 ng/ dl.

Our study showed that about 88.24% of male cases, (15) patients, had low serum testosterone level and only (2) patients, 11.76% of male cases, had normal level.

While as regard estradiol level in females, the mean level was 11.02 ±18.80.

Our study showed that about 11 cases (84.62%) had low level of serum estradiol and only 2 cases (15.38%) had normal level.

Table (4): FSH & LH levels in studied population.

Variables	Pretest	Posttest	P value
FSH Mean ±SD	1.45 ±1.88	3.78 ±4.19	<0.0001
FSH Low Normal	 18 (60.00%) 12 (40.00%)		
LH Mean ±SD	1.91 ±4.79	6.52 ±7.50	0.0001
LH Low Normal High	 15 (50.00%) 14 (46.67%) 1 (3.33%)		

Table (5): Testosterone level in males and estradiol level in females.

Variable	level	Low level (frequency	Normal (frequency)
Testosterone	17 ±0.68	15 (88.24%)	2 (11.76%)
Estradiol	11.02 ±18.80	11 (84.62%)	2 (15.38%)
Testerone/estradiol		26 (86.67%)	4 (13.33%)

As regard relation between serum ferritin levels and hormonal levels in our patients, our study showed that, as regard F.S.H level before giving GnRH analogue, 60.00% Of cases (18 cases) had low F.S.H level with mean serum ferritin was 3578.52 ng/ml. 40.00% of cases (12 cases) had normal F.S.H. level with mean serum ferritin level was 2954.58 ng/ml. Serum ferritin levels in relation to F.S.H level before giving GnRH analogue were statistically insignificance.

About relation between serum ferritin levels and L.H level before giving GnRH analogue, 50.00% of

cases (15 cases) showed low L.H level with mean serum ferritin level was 3481.20 ng/ml. 46.67% of cases (14 cases) showed normal L.H level with mean serum ferritin level was 3027.00 ng/ml. only one case showed high L.H level with serum ferritin level was 5272.00 ng/ml. Serum ferritin levels in relation to L.H level before giving GnRH analogue were statistically insignificance.

As regard relation between serum ferritin levels and estradiol levels, 84.62% of female cases (11 cases) had low estradiol level with mean serum ferritin level was 2731.38 ng/ml. Only two cases showed high estradiol level with serum ferritin was 2316.55 ng/ml. Relation between serum ferritin levels and estradiol level were statistically insignificance.

As regard relation between serum ferritin levels and serum testosterone level, 88.24% of male cases (15 cases) showed low serum testosterone level with mean serum ferritin level was 3945.20ng/ml. Only two cases had normal testosterone level. they mean serum ferritin level was 3006.00 ng/ml. Relation between serum ferritin levels and testosterone level were statistically insignificance.

After giving GnRH hormone, 9 cases showed no increase in their F.S.H level with mean serum ferritin level was 4227.40ng/ml. 14 cases 46.67% showed mild increase in F.S.H level and their mean serum ferritin level was 3142.69 ng/ml.5 cases 16.67% showed moderate increase of their F.SH level with mean of serum ferritin was 3117.24ng/ml.Only 2 cases showed very good response with mean serum ferritin level was 1118.95 ng/ml. Serum ferritin levels in relation to F.S.H level after giving GnRH analogue were statistically insignificance.

About relation between serum ferritin levels and L.H level after giving GnRH analogue, 6 cases showed no increase in their L.H level with the mean of serum ferritin level was 4841.60 ng/ml. 15 cases 50.00% showed mild increase and their mean serum ferritin level was 2712.92ng/ml. 8 cases 26.67% showed moderate improvement of their L.H level and the mean of serum ferritin level was 3106.61ng/ml.

Only one case showed very good response and the mean of serum ferritin level was 5272.00 ng/ml. Serum ferritin levels in relation to L.H level after giving GnRH analogue were statistically insignificance.

Table (6): Serum ferritin levels in relation to hormonal dysfunction.

Hormones	Levels	Number of cases	Percentage of cases (%)	Mean serum ferritin (ng/ml)	P value
Pre test					
F.S.H	Low Normal	18 12	60% 40%	3578.52 2954.58	0.46
L.H.	Low Normal High	15 14 1	50% 46.67% 3.33%	3481.20 3027.00 5272.00	0.59
Estradiol	Low Normal	11 2	84.62% 15.38%	2731.38 2316.55	0.71
Testosterone	Low Normal	15 2	88.24% 11.76%	3945.20 3006.00	0.64
Post test					
F.S.H	No Mild Moderate Good	9 14 5 2	30% 46.67% 16.67% 6.67%	4227.40 3142.69 3117.24 1118.95	0.31
L.H.	No Mild Moderate Good	6 15 8 1	20% 50% 26.67% 3.33%	4841.60 2712.92 3106.61 5272.00	0.18

4. Discussion

Iron chelation therapy is the only method of iron overload control in transfusion dependent patients. Despite the use of iron chelation therapy, the pituitary gland, peripheral endocrine tissues and gonad axis are susceptible to iron deposition and

damage**.(4).** Iron deposition on the pituitary gonadotrophic cells which is followed by disruption of gonadotropin production is the considerable reason of hypo gonadotrophic hypogonadism. Secondary hypogonadism becomes evident later in life**. (5).**

Impaired puberty in TM patients include delayed puberty, arrested puberty and hypogonadism. Delayed puberty was defined as the absence of breast enlargement in girls and testicular enlargement in boys by the age of 13 and 14 years respectively.

Arrested puberty is defined as the absence of pubertal progression for more than one year after puberty onset, where testicular volume in boys is less than 6 to 8 ml and unchanged breast size in girls. Puberty onset delay more than 2 SD beyond the mean for sex was considered as hypogonadism. **(4)**

In our study, 96.67% had not attained puberty, which included 55.17% of the boys and 44.82%of the girls (with 100% of girls having primary amenorrhea).

In study of Qazvin, Iran on 77 patients with β-thalassemia major (15-36 years old). Hypogonadism was the most common endocrine complication in 36 (46.8%) patients, 28 (36.4%) with delayed puberty and 8 (10.4%) with arrested puberty associated.(4)

Lack of puberty changes was the most common endocrine complication in a study was done in Emam Reza Hospital, Tabriz University, Iran. Eleven females were aged over 13 years and only 3 of them (27%) with a mean age of (18.71±3.9) had regular menses. Of twenty males, aged over 14 years, 6 (30%) with a mean age of (19.27±2.41) had the criteria for puberty. Overall, 22 patients (71%) had hypogonadism in this study. **(6).**

Another study in Iran, which has studied 35 thalassemia major patients (16 females and 19 males), aged between 13 and 24 years found that 60% of the patients had not attained puberty. This group included 36.8% of the boys and 87.5% of the girls (with 87.5% of girls having primary amenorrhea). **(2).**

De Sanctis and coworkers, in a study group of 238 patients, aged 2-17 years, with beta-thalassemia major, regularly followed in 13 pediatric and hematological Italian centers, found delayed puberty in 18.4% of boys and 17.7% of girls.**(7)**

In another newer study, **De Sanctis and coworkers** evaluated 3817 beta thalassemia major patients of whom thirty-six per cent of patients were over the age of 16 years; a lack of pubertal changes (40.5%) was observed in their study.**(8)**

Borgna– Pignatti and co–workers evaluated 720 thalassemia major and reported 54.7% hypogonadism in their study**. (9)**

Shamshirsaz and co–workers evaluated 258 adolescent, homozygous, beta-thalassemia patients in Tehran; impaired puberty, which occurred in approximately 77% of their patients, was the most common endocrine abnormality and hypogonadism was seen in 22.9% of boys and 12.2% of girls in their study. **(10).**

In a follow-up study by **Low L.C.** of 41 patients older than 14 years, spontaneous puberty only occurred in 32% of the patients, however a significant pubertal growth spurt with spontaneous or induced puberty was observed in 46% of these patients. **(11)**

In the present analysis, FSH levels were low in 60.00% of cases before giving gonadotropin hormone analogue which was statistically significant. LH levels were low in 50.00% before giving gonadotropin hormone analogue of cases before giving gonadotropin hormone analogue which was statistically significant.

Our study reported about 88.24% of male cases, had low serum testosterone level and 84.62% of girls had low level of serum estradiol.

In study of Rashid H. Merchant and coworkers found that FSH levels were low in 14.29%, LH levels were low in 2.86%, estradiol levels were low in 43.75% and free testosterone levels were low in 89.47% boys, however increased serum ferritin was associated with statistically significant low estradiol levels in girls.**(2)**.Similar findings were seen in a study by Ghosh et al**. 2008.**

Study on 23 female patients in London aged 13 to 29 years showed that all patients had low gonadotrophin reserves, particularly follicle stimulating hormone secretion. Most of the patients with delayed puberty showed normal gonad response to exogenous gonadotrophin (hMG), but three patients in this group had very low response, when compared with those of controls.(11)

Our study showed that,100% of cases had high serum ferritin level with the mean level of serum ferritin was 3328.94 ±2195.73 ng/ml. As regard response to G.N.R.H analogue, 70% showed increase in F.S.H levels. 80% showed increase in L.H levels.

The mean levels of serum ferritin depend on several factors, including age at presentation, age at beginning regular PRBC transfusion, age at starting iron chelation therapy, efficacy of the iron chelation drug and its compliance, and the age group of the reported series of patients. Correlation between serum ferritin with F.S.H, L.H, estradiol, testosterone before and after giving G.N.R.H analogue were statistically insignificant.

Flynn DM and coworkers found in their study that there is no direct relationship between the amount of iron accumulated and organ dysfunction**. (12)**

These results are supported by newer results in study done in 2008 where there was not significant difference in the mean serum ferritin levels for patients with delayed puberty, short stature and diabetes mellitus as compared to their counterparts without these complications**.(6,13).**

A cross-sectional analysis, followed up by the thalassemia Day Care Center (TDCC) at Kalawati Saran Children's Hospital, New Delhi, India of 214 patients showed that ferritin levels in those patients

with delayed puberty were not significantly different from those in adolescent patients with normal puberty. **(14)**

Rashid Merchant and coworkers study had postulated that it is possible that endocrine glands are extremely sensitive to iron toxicity and even small amounts of iron accumulated in the early years of life can cause irreversible damage. Interestingly, elevated ferritin levels do not always indicate iron overload state as ferritin is an acute phase reactant and gets elevated in any inflammation even in hepatitis and liver disease.**(2)**

This may explain why there is no statistically significant relationship between serum ferritin level and gonadotropin hormones levels in our study and in other studies.

Rajesh Joshi and coworkers found there was no significant statistical difference between the patients with and without endocrine abnormalities with respect to serum ferritin except for significant correlation of serum ferritin in patients with hypoparathyroidism and diabetes mellitus. (1**)**

One study explained this by there may be individual sensitivity to iron damage by other mechanisms like increased collagen deposition secondary to increased activity of the iron-dependent protocollagen proline hydroxylase enzyme, with subsequent disturbed microcirculation in the endocrine glands may operate. **(15)**

It is possible therefore, that there may be other factors responsible for organ damage. These factors include: chronic anemia, increased collagen deposition secondary to increased activity of the iron dependent protocollagen proline hydroxylase enzyme, with subsequent disturbed microcirculation in the pancreas and parathyroids, chronic liver disease secondary to iron overload and viral infections following repeated blood transfusions.(16,17)

Necropsy findings in two prepubertal patients reported by **Costin et al** showed minimal siderosis, a reduced number of primordial follicles, and thickened ovarian capsules. The magnitude of haemosiderosis overload, however, does not always parallel the degree of functional disturbance**. (18)**

Many studies explain the effect of iron on different organs as follow:with multiple transfusions, excess iron appears in the plasma that can cause progressive tissue damage in the liver, heart, endocrine glands, and other organs by generating hydroxyl free radicals, which also causes oxidative stress. Iron deposition on the pituitary gonadotrophic cells which is followed by disruption of gonadotrophin production is the considerable reason of hypogonadotrophic hypogonadism. Secondary hypogonadism becomes evident later in life. The precise mechanism whereby iron overload causes tissue damage is not completely understood, though there is evidence of free radical formation and lipid peroxidation resulting in mitochondrial lysosomal damage. So that Iron chelation therapy is the only method of iron overload control in transfusion dependent patients.(5,19,20,21)

Conclusion:

Our study supports the fact that thalassemic children have multiple endocrine dysfunctions which usually begins early in life. Therefore, regular endocrine must be carried out regularly, especially in those patients, over the age of 10 years with iron overload and poor compliance with chelating therapy. So it is recommended that patients who have ironoverload start chelating therapy during the first years of life. Because of the improved survival of thalassaemic patients, and the high incidence of multiple endocrine complications, it is important to carry out careful follow-up studies for the early detection of any other associated complications to facilitate precise treatment.

Study Limitations

The sample size is small, the cohort is young and there is limited prospective follow-up. Statistical analyses therefore have limited power, and hence our simple models may not be able to optimally identify patients at high risk of endocrinopathies. Efforts will be made to follow up these patients over a longer period of time and hopefully the results will have a more accurate predictive value.

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