Original Article ISSN (O):2395-2822; ISSN (P):2395-2814

Evaluation of Puberty and Sex Hormones Status in Multi-Transfused β-Thalassemia Major Children and their Correlation with Serum Ferritin Levels

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Received: February 2020
Accepted: February 2020

ABSTRACT

Background: β-thalassemia major is severe, transfusion-dependent anemia requiring life-long blood transfusions leading to iron overload in various endocrinal organs. Delayed puberty due to hypogonadotropic hypogonadism is one of the common endocrinal complication in multi-transfused β-thalassemia major children. This is due to impaired LH and FSH pulsatile secretions from anterior pituitary which is sensitive to increased iron concentration. **Objective:** To evaluate the pubertal status & sex hormones levels in multi-transfused children of β-thalassemia major in the age group of 11 to 19 years & correlate their pubertal and sex hormonal status with their serum ferritin levels. **Methods:** It is prospective study conducted in 50 children in the age group of 11-19 years (with serum ferritin levels >1000 µg/dL), already diagnosed with β-TM & getting repeated blood transfusions in Thalassemia Day Care Centre in the department of Pediatrics at Government Medical College, Amritsar. Detailed history and complete clinical examination with special emphasis on Sexual Maturity Rating is done. Hormonal assay (FSH and LH in both the sexes, Testosterone and Estradiol in males and females respectively) was done. Pubertal status is correlated with hormonal and serum ferritin levels. Significance is determined by p-value ($p < 0.05$). **Results:** Highest number of cases of thalassemia major was in Tanner stage-1 and Least in stage-4and 5. Delayed puberty was observed in 48% patients. Population with delayed puberty had significantly higher mean ferritin levels than that of normal ($p = 0.000$). Population with delayed puberty had significantly lower mean gonadotrophin levels (LH and FSH) than that of normal puberty ($p = 0.000$). Males and females with delayed puberty had significantly lower sex hormones levels ($p = 0.000$ and 0.016 for testosterone and estradiol respectively). There was significant negative correlation between mean serum ferritin levels and mean gonadotrophin and gonadal hormone levels of the study population ($p = 0.000$ for LH, FSH, Testosterone and 0.006 for Estradiol). **Conclusion:** Delayed puberty is a significantly frequent problem in multi-transfused β-Thalassemia Major children during adolescence and it is mainly due to hypogonadotropic hypogonadism. In the thalassemics with delayed puberty, there is evidence of more iron overload than those with normal puberty. Higher serum ferritin levels in these multi-transfused children of thalassemia major showed significantly negative correlation with their gonadotrophin and gonadal hormones levels.

Keywords: β-Thalassemia Major, multi-transfused, ferritin, delayed puberty.

INTRODUCTION

Thalassemia is a haemoglobinopathy which is characterized by decreased synthesis of one of the two types of polypeptide chains (α or β) that form the normal adult human haemoglobin molecule.

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Depending on the globin chain synthesis that is defective and decreased, α- or β– thalassemia results. The mutations in β-thalassemia are categorized as β0 and β+ mutations. The β0 mutations are considered to produce a non-functional β-globin protein or associated with absent β-globin synthesis. β+ mutations are characterized by reduced (but detectable) β-globin synthesis.[1] β-thalassemia major is the most severe form with two β-thalassemia alleles (β+/β+, β+/ β0 or β0/ β0), presenting with anemia early in life usually requiring a transfusion by the age of 2 years and remain transfusion dependent for the rest of their lives.[2] It is estimated

that >400,000 newborns are born with this disease worldwide. It is estimated that approximately 10,000 new infants are born with homozygous β-thalassemia each year in India and the vast majority of them are transfusion-dependent.[3,4]

Over the years, management of thalassemia has evolved from long term transfusions, chelation therapy and supportive care to complete cure offering near normal life. The only option for cure for homozygous thalassemia is hematopoietic stem cell transplant (HSCT).[5] But due to lack of motivation, availability of very small number of centres performing thalassemia transplant and scarcity of HLA identical donor, very few patients undergo HSCT in India. Also serious risk of short-term morbidity and occasionally mortality associated with HSCT, it is unacceptable to most parents. Transplant, although cheaper than lifelong conventional treatment of transfusion dependent thalassemia in the long-term, but is more expensive in the short-term for low socio-economic population in the developing countries. Therefore, due to free-of-cost, safe and easy availability of blood, more organized thalassemia support groups and thalassemia day care centres and availability of better chelating agents, long-term transfusion and iron chelation therapy still remains the mainstay of treatment in resource limited countries like India. Regular transfusion however, is a double-edged sword. Patients may have various complications if the transfusion is inadequate. They are also prone to complications from frequent transfusions, such as iron overload, RBC alloimmunization and transfusion transmitted infections.[6,7]

Iron overload can occur by two mechanisms in thalassemic patients. The first is through increased absorption from the intestine to provide adequate iron to the massively expanded erythron as seen in poorly transfused thalassemia patients or those with thalassemia intermedia; and the second & most important mechanism of iron overload is through regular blood (packed RBC) transfusions.[8] Also, there is no efficient mechanism for excretion of excess iron in the body in case of overload. Even well-transfused thalassemics, exhibit some increase in gut iron absorption. This is mediated by suppression of a negative iron metabolism regulatory hormone, hepcidin by ineffective erythropoisis in β-TM patients.[9]

There are direct and indirect effects of iron overload on various endocrinal organs. Indirect effects of iron toxicity are due to iron-induced oxidative stress in endocrinal organs, such as hypothalamus, pituitary and reproductive organs and due to impaired metabolism of hormones and serum anti-oxidants caused by iron toxicity on organs such as liver and pancreas.[10] The direct effect is assumed to be linked to iron accumulation in the pituitary gland, with consequent reduced volume and function, which is mostly irreversible.

The first indication of iron loading usually is the absence of the pubertal growth spurt and failure of the menarche.[11] Delayed puberty (absence of any secondary sexual characters by the age of 13 years in females & 14 years in males) is one of the common endocrinal complications in multi-transfused children of β-thalassemia major. It is mainly due to hypogonadotropic hypogonadism. The anterior pituitary is particularly sensitive to increased iron concentration, which impairs LH and FSH pulsatile secretion, leading to low or absent stimulation of the gonads, reducing the synthesis of sex hormones and the production of gametes.[12]

In developing countries like India, lack of motivation, fewer centres performing stem cell transplant, scarcity of HLA identical donors, high short-term cost of stem cell transplant for low socio-economic population, easy accessibility to blood banks, high chelation cost and poor compliance to chelation therapy precludes ideal therapy for majority of the patients. Therefore, there is possibility that there is higher prevalence of delayed puberty in children with β-thalassemia major due to iron overload caused by innumerous blood transfusions and improper chelation therapy. Hence this study was conducted on 50 indexed patients of Thalassemia Major getting regular blood transfusions at Thalassemia Day Care Centre at Bebe Nanki Mother & Child Care Centre in the department of Pediatrics, Government Medical College, Amritsar to study the frequency of delayed puberty and sex hormonal status in children of β-TM on regular transfusion therapy and their correlation, if any, with serum ferritin levels.

Aims and objectives of the study

1. To evaluate the pubertal status in multi-transfused children of β-thalassemia major in the age group of 11 to 19 years.
2. To evaluate the levels of sex hormones in these children.
3. To study the correlation of pubertal and sex hormonal status of these patients with their serum ferritin levels.

MATERIALS AND METHODS

This prospective study was conducted on 50 children in the age group of 11 to 19 years, who had been already diagnosed with β-Thalassemia Major and were on regular follow up in the Thalassemia Day Care Centre of Department of Pediatrics at Bebe Nanki Mother and Child Care Centre, Government Medical College, Amritsar.

Inclusion Criteria

- Children between age of 11-19 years already diagnosed with β-Thalassemia Major (β-TM).
- Children getting repeated blood transfusions in Thalassemia Day Care Centre in the department of

Pediatrics at Government Medical College, Amritsar.

- Children whose serum ferritin levels were above 1000 microgram/dl.

Exclusion Criteria

- Children <11 years of age and >19 years of age.
- Received in the past or receiving presently any hormonal replacement therapy like estrogen, progesterone, testosterone or Gonadotropin Releasing Hormone (GnRH) analogues.
- Children who had any clinically associated disease that could alter the pubertal status like constitutional causes, hypothyroidism and various dysmorphic syndromes etc.
- Cases of iron overload due to causes other than β-TM like hemochromatosis, porphyria cutanea tarda etc.

Methodology

A written and informed consent from the parents of all the children in the present study on a prescribed format approved from institutional ethical committee was obtained. Detailed history including age at the time of diagnosis of β-TM, frequency, number and the duration of blood transfusions received and complete clinical examination including anthropometry with special emphasis on Sexual Maturity Rating (Tanner Staging).[13] Venous blood samples were taken in the morning between 8 AM and 9 AM atleast two weeks after the previous transfusion but before the next transfusion. Values of serum ferritin were obtained from patients' medical records and the most recent value was recorded for the analysis. Hormonal assay was undertaken from the venous sample taken from the patients. Serum FSH and LH were done in both the sexes. Serum testosterone was done only in males and estradiol was done only in females. Hormonal assay was done using XEMA EIA (enzyme immunoassay) kits and their comparison to the normal values was done according to the guidelines mentioned in these kits.[14]

Reference Range of Hormonal Assays in Female Children[15]

Hormone	Age	Normal reference range
Luteinizing Hormone (LH)	9-13 years	<0.15-7.2 mIU/ml
	>13 years	1.5 - 5.6 mIU/ml
Follicle stimulating Hormone (FSH)	9-13 years	1.1 - 9 mIU/ml
	>13 years	3.6 - 7.9 mIU/ml
Estradiol	9-13 years	<10 - 55 pg/ml
	>13 years	20 - 85 pg/ml

Statistical Analysis

Demographic data was compiled for all the cases. Values of the hormonal assays and biochemical parameters were tabulated and analyzed by Student t-test. Pubertal status was correlated with hormonal and serum ferritin levels. Data is expressed as Mean ± SD. Significance is determined by p-value ($p < 0.05$).

Reference Range of Hormonal Assays in Male Children[16]

Hormone	Age	Normal reference range
Luteinizing Hormone (LH)	<11 years	1 - 5 mIU/ml
	>11 years	1.5 - 9 mIU/ml
Follicle stimulating Hormone (FSH)	<11 years	0 - 4 mIU/ml
	>11 years	0.8 - 25 mIU/ml
Testosterone	300 - 1200 ng/dl	

RESULTS

In this study, conducted in Thalassemia Day Care Centre in the department of Pediatrics, Government Medical College, Amritsar, 50 multi-transfused children having β-TM in the age group of 11-19 years were evaluated for their pubertal and sex hormonal status. As regard Tanner staging, 21out of 50 cases (42%) were in stage-1, 13 out of 50 (26%) in stage-2, 10 out of 50 (20%) in stage-3, 3 out of 50 (6%) in stage-4 and 3 out of 50 cases (6%) were in stage-5 i.e. maximum number of cases had Tanner stage-1 and minimum number had Tanner stage-4/5 [Table 1].

Table 1: Population Distribution in Different Tanner Stages

Tanner Stage	Number of cases in the study population	Percentage of the total study population
Stage-1	21	42%
Stage-2	13	26%
Stage-3	10	20%
Stage-4	3	6%
Stage-5	3	6%

On evaluating the pubertal status of the 50 multi-transfused β-TM children in this study, we found that 24 cases (48%) had delayed puberty for their age and 26 cases (52%) had normal puberty [Table 2].

Table 2: Pubertal Status of the Study Population

Pubertal status	Total patients (N)	Percentage of total study population
Delayed puberty	24	48%
Normal puberty	26	52%

While studying the medical history, it was observed that mean age at diagnosis of thalassemia major was earlier in cases of delayed puberty than those with normal puberty (19.42 ± 19.09 vs. 20.04 ± 22.09 months) but the difference was statistically not significant ($p = 0.916$). There was significant difference ($p=0.003$) between cases of delayed puberty and cases with normal puberty as regard

mean of total number of blood transfusions (326.38 ± 60 vs. 265.04 ± 76.71). Similarly, mean frequency of blood transfusions was more in population with delayed puberty than that with normal puberty (1.81 ± 0.16 vs. 1.48 ± 0.24 per month) and the difference was statistically significant (p= 0.000) [Table 3].

Table 3: Comparison of Medical History of the Population with Delayed Puberty and Population with Normal Puberty

Medical History (Mean ± SD)	Population with Delayed Puberty (Total = 24)	Population with Normal Puberty (Total = 26)	p-value
Age at the diagnosis of β-TM (months)	19.42 ± 19.09	20.04 ± 22.09	0.916
Total number of Blood Transfusions (n)	326.38 ± 60.97	265.04 ± 76.71	0.003 (significant)
Frequency of Blood transfusions (per month)	1.81 ± 0.16	1.48 ± 0.24	0.000 (significant)

Table 4: Comparison of Biochemical Parameters of the Population with Delayed Puberty and Population with Normal Puberty

Biochemical Parameters (Mean ± SD)	Population with Delayed Puberty (Total = 24)	Population with Normal Puberty (Total = 26)	p-value
Serum Ferritin (ng/mL)	5729.29 ± 1310.38	2598.65 ± 703.94	0.000 (significant)
Serum LH (mIU/mL)	1.00 ± 1.35	4.23 ± 2.53	0.000 (significant)
Serum FSH (mIU/mL)	1.16 ± 1.66	5.25 ± 2.62	0.000 (significant)
Serum Testosterone in males (ng/dL)	106.92 ± 241.79	502.66 ± 108.32	0.000 (significant)
Serum Estradiol in females (pg/mL)	19.76 ± 15.48	43.36± 15.73	0.016 (significant)

Table 5: Correlation between the Levels of Serum Ferritin and Sex Hormones

Pearson Correlation Coefficient (r) between S.Ferritin & Sex Hormones levels in the study population		
Hormones	**Serum Ferritin**	**p-value**
LH	r = -0.657	0.000 (significant)
FSH	r = -0.780	0.000 (significant)
Testosterone (in males)	r = -0.810	0.000 (significant)
Estradiol (in females)	r = -0.675	0.006 (significant)

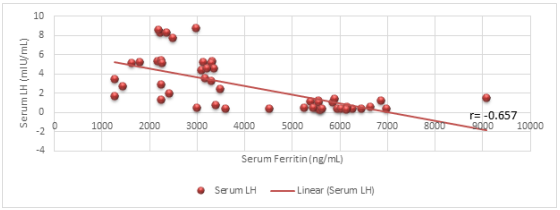

Figure 1: Scatter Diagram Showing Correlation Of Serum Ferritin Levels With Serum Lh Levels In The Study Population

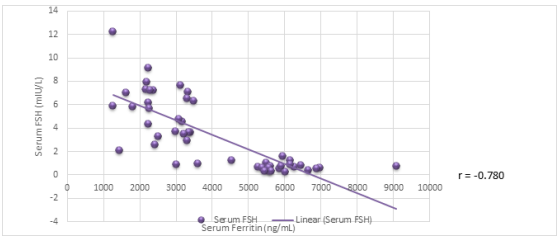

Figure 2: Scatter Diagram Showing Correlation Of Serum Ferritin Levels With Serum Fsh Levels In The Study Population

As regard biochemical parameters, mean serum ferritin levels were significantly more in delayed puberty cases than the population with normal puberty (5729.29 vs. 2598.65 ± 703.94 ng/mL ; p = 0.000); whereas, mean of different hormones was significantly lesser in population with delayed puberty than the study cases with normal puberty (LH = 1.00±1.35 vs. 4.23±2.53 mIU/mL, FSH = 1.16±1.66 vs. 5.25±2.62 mIU/mL, Testosterone in males = 106.92±241.79 vs. 502.66±108.32 ng/dL, Estrdiol in females = 19.76 ±15.48 vs. 43.36 vs. 15.73 pg/mL ; p = 0.000 for LH, FSH, Testosterone and p = 0.016 for Estradiol) [Table 4].

On correlating mean sex hormones levels with mean serum ferritin in the study population using Pearson correlation coefficient (r), it was noted that all the hormones (LH, FSH, Testosterone in males and Estradiol in females) had significantly negative correlation with mean serum ferritin levels in all the study cases (r = -0.657, -0.780,-0.810, -0.675 for LH, FSH, Testosterone and Estradiol respectively and p-value being 0.000 for LH, FSH, Testosterone and 0.006 for Estradiol) [Table 5 & Figure 1-4].

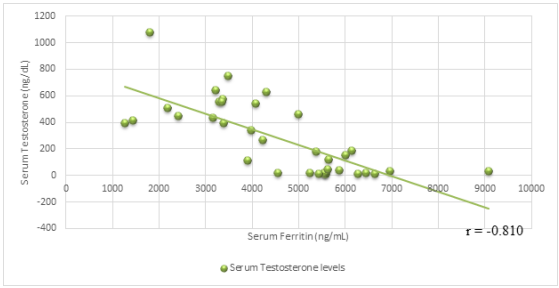

Figure 3: Scatter Diagram Showing Correlation Of Serum Ferritin Levels With Serum Testosterone Levels In The Male Study Population

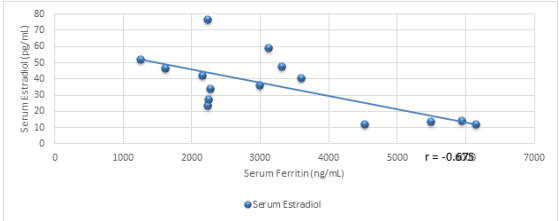

Figure 4: Scatter Diagram Showing Correlation Of Serum Ferritin Levels With Serum Estradiol Levels In The Female Study Population

DISCUSSION

Adolescent age is very crucial for the pubertal growth. Any factor causing its aberration in this age group will have deleterious effects on sexual development in the later life. Therefore, in the present study, evaluation of puberty, sex hormones status and their correlation with serum ferritin was done in 11 to 19 years age group in 50 multi-transfused children of β-Thalassemia Major registered in Thalassemia Day Care Centre of the Department of Pediatrics at Bebe Nanki Mother and Child Care Centre, Amritsar.

Although the age at which individual pubertal changes occur may vary, the timing and sequence of these changes relative to one another is predictable.[17] The progression of the pubertal development is clinically described using the Tanner stages. Kretschmer et al in their analysis of Tanner staging in 13,978 subjects (52% boys and 48% girls) in general population found that as the age advances from 11 to 14 years frequency of population increased in the latter stages of Tanner.[18] But as regard Tanner staging of the patients of β-TM in the present study, preponderance of population was seen in the initial stages of Tanner (pre-pubertal stage of Tanner constituted the maximum population of 42%, 26% population was in stage-2, 20% in stage-3 and 6% stage-4 and stage-5 each). These results were in line with the other studies done in adolescent patients of β-TM .Vahidi et al noted 36.60% cases were in stage-1, 28.20% in stage-2, 26.80% in stage-3, 5.6% in stage-4 and 2.8% in stage-5.[19] Hagag et al found 50% were in stage-1, 25% in stage-2, 13.33% in stage-3, 10% in stage-4 and 1.66% in stage-520. Thus, this preponderance of population in the initial stages of Tanner in cohort of β-TM adolescent patients in our and other studies when compared to general population can be explained that due to excessive iron loading due to multiple transfusions there is delay or failure in progression of pubertal development in patients of β-TM. There are evidences that free radical formation and lipid peroxidation can lead to the damages of mitochondrial, lysosomal and sarcoplasmic membranes.[21,22] The presence of iron deposits and oxidative damage by free radicals affects the pituitary, ovarian follicles and testis. As the result, the hypothalamic-pituitary-gonadal axis function is disturbed which eventually leads to delayed puberty.[23]

It is well documented in the literature that there is high prevalence of delayed puberty in multi-transfused children of β-TM. 48% adolescent patients of β-TM in this study had delayed puberty. These results were consistent with study done by Batubara et al in which 56% of the study population had delayed puberty.[24] Also study conducted by Hagag et al showed parallel results with prevalence of 60% in delayed puberty.[20] Similarly 51% of the population studied by Al-rimawi had delayed onset of puberty.[25] In the study done by Shamsiraz et al 76.81% of the population had delayed puberty.[5] This difference of prevalence of delayed puberty from our study may be attributed to large sample size in their study. Merchant et al found that 60% of their population had delayed puberty.[26] This difference of prevalence of delayed puberty from the current study may be due to wide range of age distribution in their study population.

In our study, we observed that there was hardly any difference between the multi-transfused cohort of the adolescent patients of β-TM with delayed puberty and those with normal puberty as far as age at the time of diagnosis of thalassemia is concerned (19.42 ± 19.09 vs. 20.04 ± 22.09 months; p = 0.916). Thus, both the cohorts i.e. cases with delayed puberty and those with normal puberty were equally susceptible to the iron overload due to multiple transfusions in future. But delayed puberty population had history of significantly (p = 0.003) higher number of transfusions than that of normal puberty cases (326.38 ± 60.97 vs. 265.04 ± 76.71) with significantly (p = 0.000) higher frequency of transfusions per month in delayed puberty cases than those with normal puberty (1.81 ± 0.16 vs. 1.48 ± 0.24). Both these factors (more frequent and more number of blood transfusions) might have made delayed puberty population prone to higher iron loading than normal puberty population.

On comparing biochemical parameters in the population with delayed puberty to that of population with normal puberty in our cohort of adolescent β-TM patients, we found that mean serum ferritin levels were significantly (p = 0.000) higher in cases with delayed puberty than those of normal puberty (5729.29 ± 1310.38 ng/mL vs. 2598.65 ± 703.94). This finding was in accordance with study done by Belhoul et al in which mean ferritin levels in group with delayed puberty were 3440.2±2062.1 ng/ml vs 2290.8±1726.6 ng/mL in group with normal puberty with statistical significance (p< 0.001).[27] In the study population of Shamsiraz et al, serum ferritin levels in the group with delayed puberty were 1407 ± 971 ng/mL. But it was not statistically significant in comparison with patients who went normally through puberty.[5] This may be explained by the lower mean ferritin levels in their study population (1441 ± 1111.3 ng/mL vs. 4101.35 ± 1885.48 ng/mL in ours). Thus, observation of higher ferritin levels in the population with delayed puberty than that of normal puberty in adolescent patients of β-TM make iron overload a risk factor for delayed puberty in these patients.

As regard comparison of hormones levels in the current study, they were significantly lower in cases with delayed puberty than those with normal puberty, p-value being 0.000 for LH, FSH, testosterone (males) and 0.016 for estradiol (females) viz mean LH = 1.00±1.35 vs. 4.23±2.53

mIU/mL, mean FSH = 1.16±1.66 vs. 5.25±2.62 mIU/mL, mean testosterone in males = 106.92±92 vs. 502.66±108.32 ng/dL and mean estradiol in females = 19.76±15.48 vs. 43.36±15.73 pg/mL. These findings were consistent with study done by Al-rimawi et al which concluded that mean LH, FSH and testosterone levels were significantly (p= 0.003 for LH, 0.02 for FSH and 0.0002 for testosterone) lower in group with delayed puberty (LH= 0.82 ±0.18 mIU/mL, FSH = 0.78±0.17 mIU/mL and testosterone = 37.2±19.9 ng/dL) than that of group with normal puberty (LH= 2.47±0.46 mIU/mL, FSH= 3.6±1.1 mIU/ml and testosterone = 248.6±43.7 ng/dL.[25]

In our study, correlation between mean levels of sex hormones and mean serum ferritin in the study population came out to be negative and it was statistically highly significant, p-value being 0.000 for LH (r = -0.657), FSH, (r = -0.780), Testosterone in males (r = -0.810) and 0.006 for Estradiol in females (r = -0.675). Results were parallel to the study done by Yenzeel et al, in which the correlation between sex hormones and ferritin was also negative (r = -0.524 for LH, r = -0.291 for FSH and r = -0.072 for Estradiol).[28] Abo-elwafa et al also inferred that there was negative correlation between serum FSH and ferritin levels (r = -0.18) but it was statistically insignificant (p = 0.33), which may be related to the smaller number of cases studied by them.[29]

CONCLUSION

It can be concluded that delayed puberty is a significantly frequent problem in multi-transfused β-Thalassemia Major children during adolescence and it is mainly due to hypogonadotropic hypogonadism. In the thalassemic patients with delayed puberty, there is evidence of more iron overload as reflected by significantly higher levels of serum ferritin in them than those with normal puberty. Higher serum ferritin levels in these multi-transfused children of thalassemia major showed significantly negative correlation with their gonadotrophin and gonadal hormones levels. Therefore, all multi-transfused thalassemics during adolescence must be subjected to thorough investigations, proper monitoring during follow-up and ensured adequate iron chelation therapy so as to keep their serum ferritin levels within the desired range. This can definitely help them to have their normal gonadal reserve and ultimately maintain their normal sexual growth.

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How to cite this article: Singh P, Dhillon SPS, Singh J, Goyal A, Singh V, Kaur N, Kaur J. Evaluation of Puberty and Sex Hormones Status in Multi-Transfused β-Thalassemia Major Children and their Correlation with Serum Ferritin Levels. Ann. Int. Med. Den. Res. 2020; 6(2):PE09-PE15.

Source of Support: Nil, **Conflict of Interest:** None declared