

The association between iron over load and tanner stage retardation in the females with B-thalassemia major in Thalassemia center of Diwaniyah maternity and children teaching Hospital.

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Abstract

Background:

Despite optimal therapy of patients with B- thalassemia major included regular blood transfusion program and iron chelation agents helped by increasing survival of these patients but remained major problem in adolescent of these patients such as growth failure and hypogonadism.

Aim of study: To determine the association between iron over load and tanner stage retardation among female patients with B- thalassemia major in Thalassemia center of Diwaniyah maternity and children teaching Hospital.

Methods: The current study was carried out on all female patients diagnosed β -thalassemia major on the base of the blood investigations (peripheral blood counts and hemoglobin electrophoresis), with their age range from 13 years to 16 years who registered in Thalassemia center of Diwaniyah maternity and children teaching hospital in Al- Diwaniyah Governorate, Republic of Iraq. The Data collection was carried out during the period from the 1st of April of 2016 to 30th of December of 2016. They were studied for determining the association between iron over load and tanner stage retardation among female patients with B- thalassemia major.

The following data were collected from the patients:

Name, age, address, age of diagnosis of thalassemia, number of blood transfusion per year, types of chelating agent.

In the physical examination, the patients were assessed for weight, height, Tanner stages and body mass index (BMI) which recorded.

S. Ferritin value was used to assess iron load, pelvic ultrasound was checked to assess the size of uterus and both ovaries.

Results: Total numbers of B- thalassemia major female patients are **31** patient, aged **13-16 years (mean age: 14.13 \pm 1.20)**. Age of patients at time of diagnosis of B- thalassemia major range from **0.17 to 5** year with **Mean \pm SD (1.40 \pm 1.30)**. Frequency of Blood transfusion (time/Year) range from **6 to 33** time/Year with **Mean \pm SD (16.68 \pm 4.98)**. Level of serum ferritin of the patients was ranged from **913-12000 ng/ml** with **Mean \pm SD (4963.60 \pm 3580.39)**.

Tanner stage **I** was predominant accounting for **87%** whereas stage **II** and **III** accounted for **10%** and **3%** respectively. There was no significant correlation between Tanner stage and age of patient and also no significant correlation with age at time of diagnosis ($P > 0.05$). There was a significant negative correlation between frequency of blood transfusion and Tanner ($r = -0.385$, $P = 0.045$), so that the higher the frequency of transfusion, the lower the tanner stage is. No correlation was found between the dose of chelating agent and Tanner stage ($P = 0.599$). Height and weight of patients were significantly correlated with Tanner stage ($P = 0.028$ and $P = 0.007$, respectively). No significant correlation was found between serum ferritin and Tanner stage ($P = 0.444$). There was a significant correlation between Uterus size, ovarian size and Tanner stage ($P = 0.007$ and $P = 0.007$, respectively).

Conclusion: These results showed that no significant correlation between tanner stages retardation and iron overload in female patients with β -thalassemia major measured by serum ferritin. Because of inflammation falsely increase serum ferritin or because of the relationship between body iron and level of serum ferritin is not always within the linear range especially in condition of inflammation or tissue damage. So that serum ferritin level is not a reliable indicator of total body iron stores in patients with thalassemia major, therefore; we needed another indicator to measure total body iron stores in patients with thalassemia major such as the liver iron concentration.

Key Words: Tanner stages of female patients with β -thalassemia major, serum Ferritin , pelvic ultrasound examination to assess the size of uterus and both ovaries.

Introduction

Thalassemia is an inherited autosomal recessive disorder of haemoglobin synthesis characterized by defecting of globin chain production , leads to either a complete absence of β - globin chain production (β^0 - thalassemia), or a partial reduction (β^+ thalassemia).^(1,2) B-thalassemia major is mostly diagnosed during the first few months of life, when the fetal haemoglobin (HbF) level is decreased. The usual symptoms of anemia like fatigue, lethargy, and pallor are present.⁽³⁾

Patients with β - thalassemia major need regular transfusion of red cells each 2-5 weeks for long life to keep adequate hemoglobin levels (the pre- transfusion Hb – level above 9-10.5 gm/dl & post transfusion Hb should not be more than 14-15 gm/dl) ,obtain normal growth and development and suppress the erythroid hyperplasia and skeletal deformities. Before starting blood transfusion therapy, a red cell phenotype is required; blood products that are leukoreduced and phenotypically matched for the Rh antigens are obtained for transfusion. This prevents unnecessary infusion of plasma proteins and white cells and thus prevents non hemolytic febrile transfusion reactions.⁽⁴⁾

Iron overload results from two sources firstly from transfused blood (each unit of packed red cells provides 200-240 mg of iron),and secondary from enhanced gastrointestinal tract iron absorption. The body iron stores present saturated after receiving approximately 20-30 blood transfusions (500

mg iron/Kg). Increased iron accumulation after this level will lead to iron accumulation in many organs of the body especially the liver , heart and endocrine organs leading to apparent signs of organ damage.^(4,5)

Patients with β - thalassemia major who are receiving transfusion therapy also require iron chelation agent such as desferrioxamine (Desferal, DFO) administered as intravenous infusion or slow subcutaneous through a portable pump at a dose of 30-60 mg/kg/day over 8-12 hr., for 5-6 days/week. About 8 mg of iron is bound by 100 mg of desferrioxamine. Side effects of desferrioxamine are often irritation at sites of the injection and febrile reaction, the dangerous side effect is infection with *Yersinia enterocolitica* and severe mucormycosis. Long term toxicities include auditor toxicity and ocular toxicity. Another iron chelating agent is Deferasirox (Exjade, DFX) administered orally at dose of 20 – 40 mg / kg once daily received before the breakfast. Side effects of Deferasirox are often gastrointestinal upset, increased creatinine and increased hepatic enzymes. *other* orally iron chelating agent is Deferiprone also administered orally at dose of 75mg/kg divided into three sub doses, each given one hour before food. Adverse effects of deferiprone include agranulocytosis, arthropathy which necessitates discontinuation of the therapy. Gastrointestinal intolerance, zinc deficiency and fluctuation of liver enzymes are other side effects. The Purpose of chelation therapy is to

maintain level of body iron on safe side at all time (serum ferritin is 500-1000 microgram /liter, or linear iron concentration between 4 - 7.5 mg/gm dry weight) ^(6,7) .
(8)

The Tanner staging system for female that published by Marshall and Tanner and the sequence of changes, is presented below.

Girls - breast development

Stage 1: B1:Prepubertal

Stage 2: B2:Early puberty (breast bud stage (thelarche): Breast and papilla elevated as small mound; diameter of areola increased

Stage 3: B3: Mid puberty (breast and areola enlargement; no contour separation)

Stage 4: B4:Advanced puberty (areola and nipple project separately from the contour of breast)

Stage 5: B5:Adult(Mature stage: fully developed breast, nipple projects, areola part of general breast contour)



Figure 1:-Tanner stages (1-5) of breast changes in Adolescent females.

girls - pubic hair

- Stage 1: PH1:Prepubertal (can see velus hair similar to abdominal wall)
- Stage 2 PH2: Early puberty (Sparse growth, lightly pigmented hair, straight, at along medial border of labia).
- Stage 3: PH3: Mid puberty (Darker, beginning to curl, increased amount and extends over pubic junction)
- Stage 4: PH4: Advanced puberty(Hair Coarse, curly, abundant, corresponds to adult growth, but covering smaller area than in adult; no spread to medial surface of thighs)
- Stage 5: PH5:Adult in type and quantity, with feminine triangle, spread to medial surface of thighs .

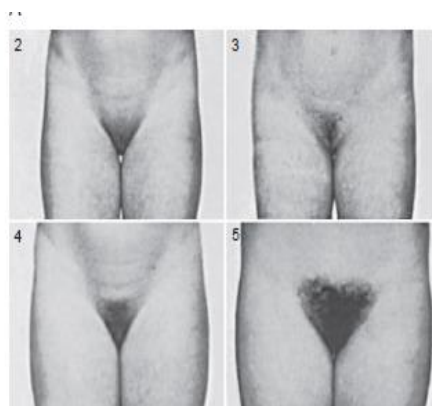


Figure 2:-Tanner stages (2-5) of pubic hair changes in adolescent and females.

Puberty retardation and hypogonadism are considered the common endocrine complications in patients with β - thalassemia major due to iron over load leads to damage to the hypothalamic-pituitary axis. Delayed puberty is known as no sign of puberty in females by 13 years and in males by 14 years. Hypogonadism is known as disappear of testicular development in males and breast development in females by 16 years).⁽⁹⁾

Regular fallow up to the patients with β - thalassemia major for delayed puberty and hypogonadism is important to start therapy and avoid complications. Tanner staging each 6 months for preadolescent patients and yearly assessment of luteinizing hormone ,follicular stimulating hormone, insulin-like growth factor (IGF), and IGF-binding protein-3 for patients

with age from 8to10 years are required. Gonadal steroids (ethinyl estradiol) must be started for girls with age more than 13 years not appearing pubertal signs as orally administrated(2.5-5ug daily)for six months then monitoring of hormonal investigations. if the puberty signs does not present within six months after complete the treatment, starting with oral oestrogen (ethinyl estradiol) with increasing dosages from 5 to 10ug daily for another 12 months .if uterine bleeding does not happen, low oestrogen –progesterone hormone therapy is the required treatment.⁽¹⁰⁾

Aim of the study:-

This study was clarified to determine the association between iron over load and tanner stage retardation among female patients with B- thalassemia major in Thalassemia center

of Diwaniyah maternity and children teaching Hospital.

The association of the female tanner stages with certain variable factors including age of the patients , age of diagnosis of B-thalassemia, number of blood transfusion , serum ferritin , pelvic ultrasound examination to assess the size of uterus and both ovaries.

Patients and Methods

The current study was carried out on all female patients diagnosed β -thalassemia major on the base of the blood investigations (peripheral blood counts and hemoglobin electrophoresis). Total numbers of B-thalassemia major female patients are **31** patient, with their age range from 13 years to 16 years who registered in Thalassemia center of Diwaniyah maternity and children teaching hospital in Al- Diwaniyah Governorate, Republic of Iraq. The Data collection was carried out during the period from the 1st of April of 2016 to 30th of December of 2016. They were studied to determine the association between iron over load and tanner stage retardation among female patients with B-thalassemia major.

All these patients were treated with frequent blood transfusion depending on hemoglobin's level and chelating agent in daily does adjusted according to ferritin level.

The questionnaire and data collection:

1- The information were taken from the patients or their families (mother ,father) and the card visit. Data include :age, address, age at diagnosis of thalassemia, Frequency of blood transfusion per year, types of the chelating agent

2-Assessment of stages of Tanner stage (breast size and pubic hair) by the researcher based on the Tanner staging system for female that published by Marshall and Tanner.

3-measurements such as height was measured using an age appropriate stadiometer and weight was measured by weight scale.

4-Laboratory Investigation: Serum ferritin level was used to assess iron load, pelvic ultrasound was checked to assess the size of uterus and both ovaries.

Statistical analyses

Statistical analysis was analyzed by using SPSS (statistical package for social sciences) version (16) computer soft ware of Excel 2007. A level less than 0.05 was considered as statistically significant.

Results

General characteristics of the study sample

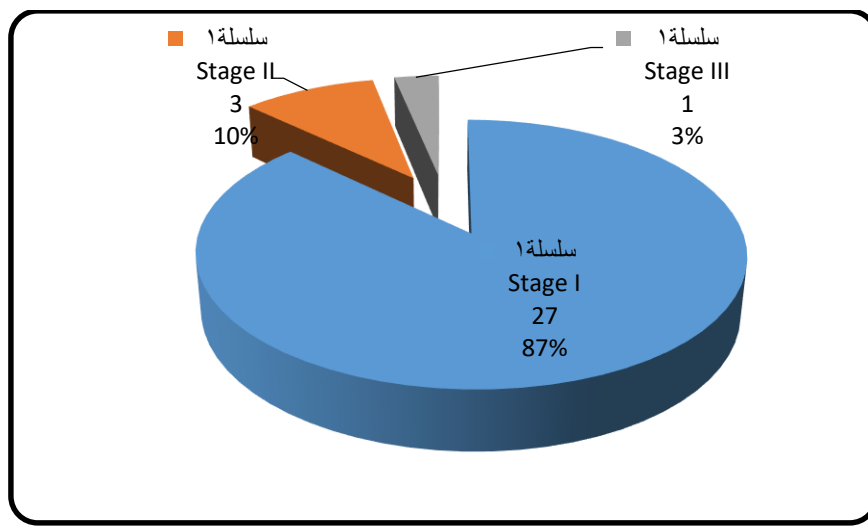
Total numbers of B- thalassemia major female patients are **31** patient, aged **13-16 years (mean age: 14.13 \pm 1.20)** . Age of patients at time of diagnosis of B- thalassemia major range from **0.17 to 5 year** with **Mean \pm SD(1.40 \pm 1.30)**. Frequency of Blood transfusion (time/Year) range from **6 to 33 time/Year** with **Mean \pm SD(16.68 \pm 4.98)**. The female patients with B- thalassemia major were taken Dosage of chelating agents range from **30 to 40 mg/kg/day** with Mean \pm SD (37.42 \pm 3.62).

Weight, height and body mass index(BMI) of patients were **22-55 kg, 122-155 cm, 13.02 - 25.25 kg/m² respectively**. With Mean \pm SD 34.64 \pm 8.15, 136.10 \pm 8.38, 18.57 \pm 3.26 respectively. Level of serum ferritin of the patients was ranged from **913-12000 ng/ml** with **Mean \pm SD (4963.60 \pm 3580.39)**. Uterus and Ovarian sizes of the patients measured in (mm) by pelvic ultrasound were **18.00 -57.00mm , 12.00 - 36.00mm** respectively with **Mean \pm SD 27.35 \pm 9.98, 16.39 \pm 6.78** respectively. General characteristic of the study sample are shown in **table 1**.

Table 1: General characteristic of the study sample

Characteristic	Mean \pm SD	Range
Age (year)	14.13 \pm 1.20	13.00 -16.00
Age at time of diagnosis (year)	1.40 \pm 1.30	0.17 -5.00
Frequency of Blood transfusion (time/Year)	16.68 \pm 4.98	6.00 -33.00
Dose of chelating agents (mg/kg/day)	37.42 \pm 3.62	30.00 -40.00
Weight (kg)	34.64 \pm 8.15	22.00 -55.00
Height (cm)	136.10 \pm 8.38	122.00 -155.00
BMI (kg/m ²)	18.57 \pm 3.26	13.02 -25.25
Serum ferritin (ng /ml)	4963.60 \pm 3580.39	913.00 -12000.00
Uterus size (mm)	27.35 \pm 9.98	18.00 -57.00
Ovary size (mm)	16.39 \pm 6.78	12.00 -36.00

Tanner stage **I** was predominant accounting for **87%** whereas stage **II** and **III** accounted for **10%** and **3%** respectively as show in figure 3.

**Figure 3: Tanner staging**

There was no significant correlation between age of patient and Tanner stage and also no significant correlation between age at time of diagnosis ($P>0.05$). There was a significant negative correlation between

frequency of blood transfusion and Tanner ($r=-0.385$, $P= 0.045$), so that the higher the frequency of transfusion, the lower the tanner stage is. No correlation was found between the dose of chelating agent and Tanner stage

($P=0.599$). Height and weight of patients were significantly correlated with Tanner stage (**$P=0.028$ and $P=0.007$, respectively**). No significant correlation was found between serum ferritin and Tanner stage ($P=0.444$). There was a significant correlation between Uterus size, ovarian size and Tanner stage (**$P=0.007$ and $P=0.007$, respectively**). These results were shown in **Table 2**

Table 2: Correlation between Tanner staging and other variables

Parameter	r	P-value
Age	-0.035	0.854
Age of Diagnosis	-0.213	0.251
Transfusion frequency	-0.385	0.045
Dose of chelating agent	-0.098	0.599
Weight	0.395	0.028
Height	0.473	0.007
BMI	0.245	0.184
Serum ferritin	0.143	0.444
Uterus size	0.478	0.007
Uterus size	0.478	0.007

r: correlation

Discussion

The present study was performed to assess the association between Tanner stages and iron overload in B- thalassemia major female patients and correlated with different variable.

The results of our study showed that height (short stature) and weight (weight failure) of patients were significantly correlated with Tanner stage ($P=0.028$ and $P=0.007$, respectively). Our results are similar to other studies done in other areas of world like

Heshmat Moayeri MD study in Tehran, Iran⁽¹¹⁾ and M. G. Vogiatzi study in North America⁽¹²⁾. There are multifactorial pathogenesis explained growth failure in thalassemia and commonly iron overload due to frequent blood transfusion and iron toxicity on endocrine gland (Puberty retardation , hypogonadism ,GH deficiency, hypothyroidism and diabetes),Side effect of chelating agents especially desferrioxamine, Other factors like chronic anemia, hypersplenism and Folate deficiency, Calcium and zinc deficiency.⁽¹³⁾

There was a significant negative correlation between frequency of blood transfusion and Tanner ($r=-0.385$, $P= 0.045$), so that the higher the frequency of transfusion, the lower the tanner stage is. Also there was a significant correlation between Uterus size, ovarian size and Tanner stage (**$P=0.007$ and $P=0.007$, respectively**). These results were shown in **Table 2**. These result are consistent with Sutay NR study in Australia⁽¹⁴⁾ and De Sanctis V study in Indian⁽¹⁵⁾. Chronic blood transfusions is the most common cause of iron overload in B- thalassemia major leads to iron deposition on gonadotrophic cells result in failure of gonadotrophin production. Small size of uterus and ovaries resulted from hypogonadotropic hypogonadism or from deposition of iron in the ovaries itself which lead to pubertal failure. This fact One unit of blood transfused includes about 250 mg of iron. 25 units of blood transfused to the patient per year leads to accumulate 5 grams of iron per year without of chelating therapy. The deposition of iron and oxidative damage by free radicals affects the pituitary and ovarian follicles, result in dysfunction of hypothalamic- pituitary-gonadal axis which leads to pubertal failure.

No significant correlation was found between serum ferritin and Tanner stage ($P=0.444$). As shown in **Table 2**. Because of inflammation falsely increase serum ferritin or because of the relationship between body iron and level of serum ferritin is not always within

the linear range especially in condition of inflammation or tissue damage. So that serum ferritin level is not a reliable indicator of total body iron stores in patients with thalassemia major, therefore; we needed another indicator to measure total body iron stores in patients with thalassemia major such as the liver iron concentration

,this result correlate with Adamkiewicz study⁽¹⁷⁾.

Conclusions

In summary, in our study we tried to clarify the association between tanner stages retardation and iron overload in female patients with β -thalassemia major.

Our results showed that no significant correlation between tanner stages retardation and iron overload in female patients with β -thalassemia major measured by serum ferritin. Because of inflammation falsely increase serum ferritin or because of the relationship between body iron and level of serum ferritin is not always within the linear range especially in condition of inflammation or tissue damage. So that serum ferritin level is not a reliable indicator of total body iron stores in patients with thalassemia major, therefore; we needed another indicator to measure total body iron stores in patients with thalassemia major such as the liver iron concentration.

Recommendation

- 1- In patients with iron overload due to frequent blood transfusion, repeated measurements of the liver iron concentration can give a quantitative reading of the long-term iron balance.
- 2- Serum ferritin level is not always indicator of body iron concentration because of inflammation falsely increase serum ferritin or because the relationship between body iron and level of serum ferritin is not always within the linear range especially in condition of inflammation or tissue damage.

- 3- Regular follow up to the patients with β -thalassemia major for delayed puberty and hypogonadism is important to start therapy and avoid complications. Tanner staging each 6 months for preadolescent patients and yearly assessment of luteinizing hormone, follicular stimulating hormone, insulin-like growth factor (IGF), and IGF-binding protein-3 for patients with age from 8 to 10 years are required.
- 4- Gonadal steroids (ethinyl estradiol) must be started for girls with age more than 13 years not appearing pubertal signs as orally administered (2.5-5ug daily) for six months then monitoring of hormonal investigations. if the puberty signs does not present within six months after complete the treatment, starting with oral oestrogen (ethinyl estradiol) with increasing dosages from 5 to 10ug daily for another 12 months. if uterine bleeding does not happen, low oestrogen –progesterone hormone therapy is the required treatment.

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