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Full Length Research Paper

Comparison of some complication in β-thalassemia patients with control group

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Background and Objectives: Beta thalassemia is a type of inherited blood disorder, characterized by reduced or absence of synthesis of the beta chains. Currently, blood transfusion and sufficient of iron chelation therapy are important factors for treatment and follow up of thalassemia patients. Fortunately, high blood transfusion patients cause progressive iron overload. Consequently, the excess iron is deposited as hemosiderin and ferritin tissues and multiple complications such as liver, heart, endocrine dysfunction like hypothyroidism and hypogonadism. Aims: In comparison of the serum T4, TSH, estradiol, testosterone and vitamin D levels of ß-Thalassemia major with control group. Method: Thirty-eight patients with β-Thalassemia major with mean age of 14.08± 3.02 years were studied. All cases received blood transfusion and given chelation therapy. Also, thirty-eight healthy persons with mean age of 13.34± 2.74 years participated in the control group. Result: In comparison of β- thalassemia major patients with control group, the results indicated the serum level of FT4 hormone did not differ significantly from the two groups whereas TSH (3.86± 2.7 1 µlU/ml) in the β-thalassemia major patients were increased significantly (p<0.05) compared with control group (2.72± 1.01 µlU/ml) and 23.68% (9/38) had subclinical hypothyroidism. Estradiol level in β-thalassemia major patients was significantly different (p< 0.01) from control group. The level of estradiol in the patients (30.60± 14.68 pg/ml) is high significant (p< 0.01) decreased that compared with level in control group (13.83 ± 9.06 pg/ml) more than 13years. Mean level of testosterone was highly significantly lower in β-thalassemia major patients than control group (p< 0.001). The mean of testosterone level was 1.22± 0.83 ng/ml in β-thalassemia patients and3.71± 1.32ng/ml in control group more than 14 year. Mean of 25-hydroxy vitamin D level (11.11 ± 4.36 ng/ml) in patients was significantly lower than control group (14.03± 5.96 ng/ml). Conclusion: These results indicate high prevalence vitamin D deficiency, hypothyroidism and defect the puberty. Oral vitamin D or fortified milk with vitamin Suggested for maintain adequate level of vitamin D that has important role for balance of calcium and bone growth. Thyroid drug, estrogen and testosterone supplementation is a safe for thalassemia patients have each types of endocrine disorders.

Keywords: β-thalassemia, T4, TSH, estradiol, testosterone and vitamin D

INTRODUCTION

Thalassemia is a basic common genetic disorder worldwide (Cao and Galanello, 2010). Most of these patients are borned in developing and low-income countries, specially; affecting individuals originate from Mediterranean, Middle East, Asian subcontinent and Southeast Asia (Weatherall and Clegg, 2001 and Cao and Kan, 2013). Thalassemia has been studied in various cities in Iraq and different fields of study (Al-Samarrai *et al*., 2008; Abdulzahraa *et al*., 2011 and Al-Hakeim *et al*., 2015).

β-Thalassaemia major is a type of chronic, inherited and microcytic anemia which is characterized by impaired

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biosynthesis of the β-globin chain of hemoglobin that lead to accumulation of unpaired α-globin chain. Excess of the α-globin in chains lead to impaired erythropoiesis and is the primary reason for the cellular oxidative damages and irons overloading (Mahdi, 2014).

Blood transfusion and iron chelation therapy have enhanced the quality of life and life-span for about 30 years (Telfer *et al*., 2009). Unfortunately repeated blood transfusions and increased gastrointestinal iron absorption lead to progressive iron overload in various tissues of the patients with β-thalassemia major disease (Bhat et al., 2013). The progressive iron overload causes ineffective erythropoiesis, gastrointestinal absorption of iron and lack of physiologic mechanism for excreting excess iron which results in hemochromatosis (Sagare and Trivedi, 2014). Iron is deposited as hemosiderin and ferritin in the liver, spleen and some endocrine gland. Consequently, may develop endocrinal complications such as growth retardation, failure of sexual maturation, diabetes mellitus, insufficiency of the parathyroid, thyroid, pituitary, and less commonly adrenal glands (Borgna–Pignatti and Galanello, 2004 and Galanello and Origa, 2010).

The frequency of hypothyroidism in β-thalassemia patients ranges from 6-30% among different countries depending on chelation regimens (De Sanctis *et al*., 2004).

Thyroid dysfunction was defined as follows: Overt hypothyroidism (low free thyroxine [FT4] and increased thyroid-stimulating hormone [TSH] levels >5μIU/ml); subclinical hypothyroidism (normal level to the FT4 and TSH between 5-10 μIU/ml) and central hypothyroidism (low FT4 and normal or decreased TSH) (Soliman *et al*., 2013).

All studies have shown that the prevalence of hypogonadism in the adult β-thalassemia patients is very high (Tiosano and Hochberg, 2001; Merchant *et al*., 2011 and Sharaf *et al*., 2014). Impaired puberty in β-thalassemia patients include delayed puberty, arrested puberty and hypogonadism. Delayed puberty is defined as the absence of breast development in girls and testicular enlargement in boys between the ages of 13 and 14 years respectively. Arrested puberty is defined as the absence of pubertal development for more than one year after puberty onset, where testicular volume in boys is less than 68 ml and unchanged breast size in girls (Kyriakou and Skordis, 2009).

Multiple studies emphasized high prevalence of vitamin D deficiency that occurs in children and adolescents with β-thalassemia major may contribute to variety of bone disorders including bone pain or deformity, bone age delay, growth failure, rickets, scoliosis, spinal deformities, nerve compression and pathologic fracture such as osteopenia or osteoporosis (Saffari *et al*., 2012; Soliman *et al*., 2013 and Fahim *et al*., 2013). Adequate level of vitamin D is important for optimum skeletal health and reducing fracture risk (Soliman *et al*., 2013). Hence, vitamin D is an important role for facilitating calcium metabolism and bone mineralization; is helpful for phosphate, magnesium metabolism and promote calcium absorption (Casey *et al*., 2010). Furthermore, this is established that without vitamin D, only 10–15% of dietary calcium and about 60% of phosphorus is absorbed (Bouillon, 2001; De Luca, 2004 and Holick and Garabedian, 2006).

Vitamin D level is most often determined by measuring 25-hydroxyvitamin D (25[OH] Vitamin D) in serum or plasma. This form of the vitamin is considered to best reflects the body supply (Zittermann, 2003). Most experts agree that 25[OH] Vitamin D of < 20 ng/ml is considered to be vitamin D deficiency whereas a 25(OH) Vitamin D of 21-29 ng/ml is considered to be insufficient (Holick, 2009).

Materials and methods

The study was undertaken in thalassemia center in Duhok from 1st November, 2013 to 30th September 2015. Thirty- eight patients with β- thalassemia major were selected with the mean age 13.34±2.7. All patients were under regular transfusion program (200 ml packed RBCs/kg, at 4-5 weeks interval) with the aim of maintaining pre-transfusion hemoglobin (Hb) levels above 9 g/dl. Additionally, all patients received desferrioxamine or desferal and folic acid supplements. Also, thirty-eight (mean age 14.08± 3.02) healthy persons participated in the present study as the control group with their age range comparable to that of the patients. None of healthy persons have anemic or manifested an evident systemic disease.

Blood samples were collected from β-thalassemia patients and healthy control group. Samples were taken from patients prior to the blood transfusion to avoid possible measurement of exogenously transfused hormones. Then blood samples were transferred into labeled tubes and centrifuged. Serum was collected for further analysis in polythene tube and stored at –20 ^{0}C.

Level of the serum FT4, TSH, estradiol and testosterone and Vitamin D were studied by enzyme linked fluorescent assay method employing VIDAS instrument (Biomerifux, France). The Estradiol and testosterone level test were correlated with the pubertal stage of the patient and control persons.

Statistical analysis

Data were analyzed by GraphPad Prism 5 by using t test. β-thalassemia major patients were compared with control group. Results were expressed as mean ± standard deviation and P-values < 0.05 were considered statistical significant and pointed as *****.

Results

Table (1) shows the different levels of parameters

Table 1. Shows the comparison levels of parameters between β-thalassemia patients and control group

Parameters	Control Mean ± SD	β-Thalassemia Mean ± SD	P-value
Age (years)	13.34± 2.74	14.08± 3.02	NS
FT4 (pmol/l)	13.13 ± 1.23	13.47± 1.56	NS
TSH (µIU/ml)	2.72± 1.01	3.86± 2.7 1	p <0.05
Estradiol (pg/ml)	30.60± 14.68	13.82± 9.06	p <0.01
Testosterone (ng/ml)	3.71± 1.32	1.22± 0.83	p<0.001
25-hydroxy vitamin D ng/mL	14.03 ± 5.96	11.11 ± 4.36	p <0.05

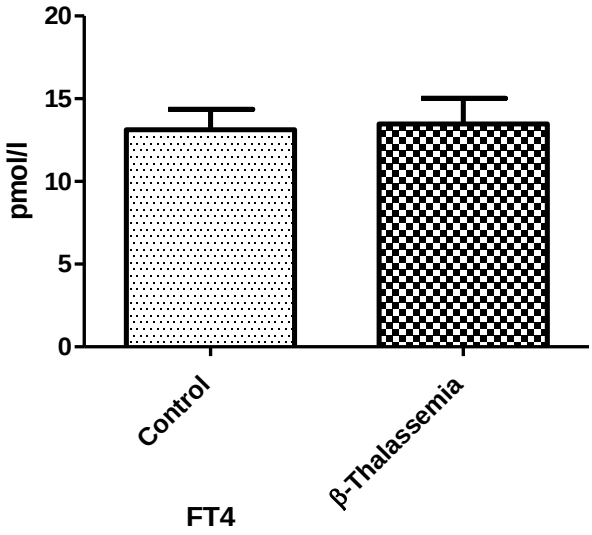

Figure 1. Comparison the average level of FT4 in the patients and control group.

between β-thalassemia major patients and control group. The two groups had no significant differences in ages.

Figure (1) indicates the comparison of FT4 assay in the patients and control group. The mean FT4 level was 13.47±1.56 pmol/l in β-thalassemia major patients and 13.13 ± 1.23 pmol/l in control group. There was no significant variation in the levels of this hormone compared between patients and control group.

Figure (2) represents the comparison of TSH level in patients and control group. The mean TSH levels were 3.86± 2.7 1 µIU/ml in patients and 2.72± 1.01 µIU/ml in control group. TSH levels were significantly higher in patients as compared with control group (p <0.05) and 23.68 percentage (9/38) from β-thalassemia major patients had elevated TSH levels that indicating primary subclinical hypothyroidism.

Figure (3) Comparison of estradiol assay in patients and control group with female ages more than 13. The mean levels were 30.60± 14.68 pg/ml in β-thalassemia patients and 13.82± 9.06 pg/ml in control group. Mean levels of estradiol were significantly lower in β-thalassemia major patients than in control group (p< 0.01) and 64.28 percentage (9/14) from the β-thalassemia patients have low level of the estradiol comparison the normal level of esrtadiol

Figure (4) shows the mean testosterone levels were 1.22± 0.83 ng/ml in patients and level in the control group 3.71± 1.32 ng/ml more than 14 years. There was highly significant decreases in the mean of serum testosterone in β-thalassemia major patients than control group (p< 0.001) and 77.77 percentage (7/9) from the patients has low level from the normal value.

Figure (5) shows the analysis of serum 25-OH vitamin D. The mean levels of vitamin D were 11.11 ± 4.36 ng/ml in patients and 14.03 ± 5.96 ng/ml in control group. Statistically, this difference was significant between patients and control group and 94.74 Percentage (36/38)

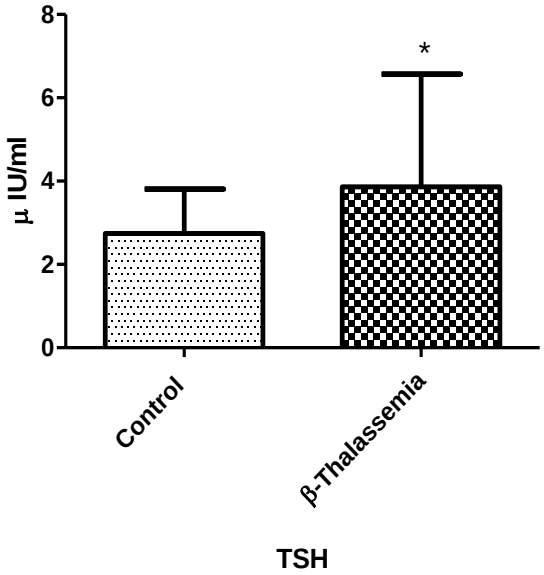

Figure 2. Comparisons of TSH levels among different study groups.

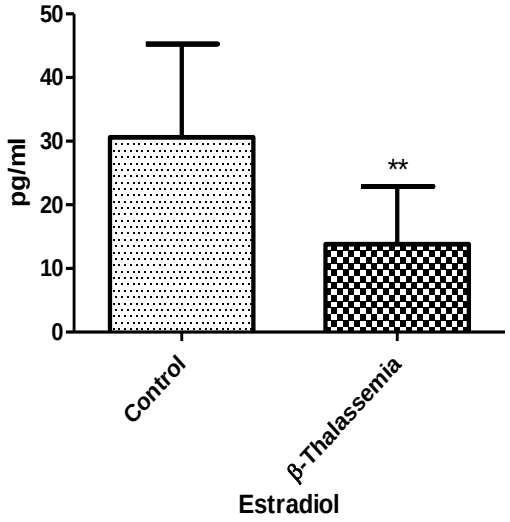

Figure 3. Comparison the mean level of the Estradiol in the patients and control group.

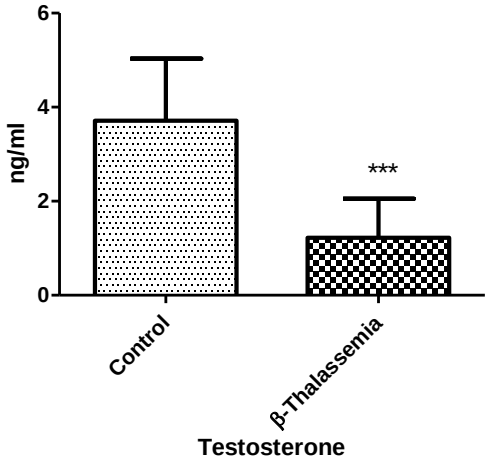

Figure 4. Different levels of testosterone in β-thalassemia patients and control group.

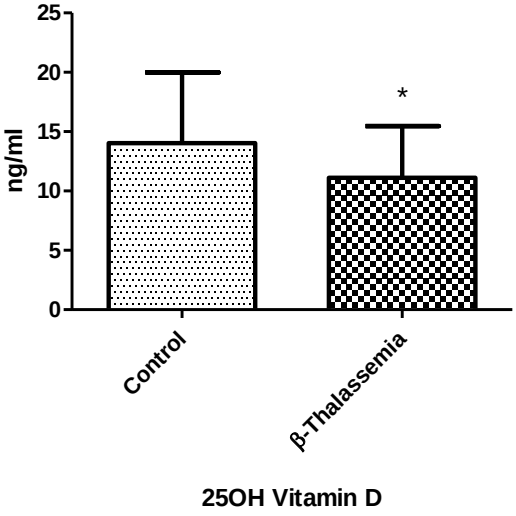

Figure 5. 25 OH Vitamin D level in β-thalassemia patients and control group expressed as mean ± standard deviation

of patients have vitamin D deficiently that compared with 78.94 percentage (30/38) control group.

Discussion

Iron overload due to multiple transfusions in thalassemia major patients is the main cause of complications, such as thyroid dysfunctions are well documented in patients with thalassemia major requiring frequent and recurrent blood transfusions (Berkovitch *et al*., 2000).

Our study showed that FT4 levels were in normal range in all patients compared with control group, whereas TSH level in patients were significantly higher than control group.

Jain (1994) reported that in the β-thalassemia patients, the mean serum total T4 and T3 levels were significant lower ($p < 0.001$) and TSH level was higher than control group. Other study was demonstrated significant ($p<0.05$) increased TSH (3.5 ± 1.7 µIU/ml) in the thalassemia patients, when compared with healthy control group (2 ± 1.2 µIU/ml), those results showed 20% with subclinical hypothyroid (Salih and Al-Mosawy, 2013). Whereas other study showed that the serum level of T4 was lower in the

β-thalassemia patients, when compared to the control group and TSH level in the patient group was higher than the control group (Asad *et al*., 2016).

Our studies have shown hat 23.68 percentages from β-thalassemia patients had subclinical hypothyroidism, which was in good agreement with the study by Malik et al., (2010). Additionally, our finding disagrees with Mula-Abed et al., (2008) Pirincciogla et al., (2011) that the prevalence was 3.3%. Recent study by Ayoub et al., (2016) demonstrated prevalence of hypothyroidism 21.6 % of β-thalassemia patients in Baghda (high TSH level with normal T4) in their study subjects. Even so, secondary hypothyroidism was rare in β-thalassemia patients, which was not observed. Ghosh, (2008) reported the prevalence of Subclinical hypothyroidism about 23.5% in West Bengal. In one study was shown that Progressive worsening of thyroid function observed in 35% of β-thalassemia patients have the age 18. The lack of proper increase of TSH in response to low levels of T4 in those patients that indicated a high incidence of defective pituitary thyrotrophic function (Soliman *et al*., 2013).

However, in the fact the difference of incidence may be linked on the age of the study population, duration of blood transfusions, ferritin levels and dose of the iron-chelating agent (Aydinok *et al*., 2002 and Zervas *et al*.,2002). In this study hypothyroidism was found in 23.6% of β-thalassemic patients depending on the elevated of thyroid stimulating hormone more than 5 μIU/ml. The high prevalence ranges our study can be attributed to the fact that most of our patients of β-thalassemia major were in the second decade. Many mechanisms responsible for thyroid dysfunction in β-thalassemia patients have been suggested but the exact mechanism is not known. Hypothyroidism may be related to the increase of iron in thyroid glands due to blood transfusion by iron overload leading to gland dysfunction (Fung *et al*., 2006). Consequently, the iron stored as ferritin is deposited in organs as hemosiderin, a toxic substance affecting tissues at least partially by inducing oxidative stress (Shizukuda *et al*., 2007). Additionally, formation of free radical and lipid peroxidation was causing damage of the mitochondrial, lysosomal, sarcolemma membrane and DNA (Lekawanvijit and Chattipakorn, 2009 and Hershko, 2010). Those damages involve practically all organs in the body like spleen, liver and bone narrow. Also, iron poisoning effects on several endocrine glands as the thyroid gland function and gonads (Berkovitch et al., 2000).

Most thalassemia patients have a delayed or absent puberty occur with appearance disorder in menstrual cycle and anovulation in women. Also, happen abnormalities spermatic and reduced sexual activity in males. Frequency of failure in onset of puberty is 50%in some studies and may approach even 100% (Tiosano and Hochberg, 2001 and Moayeri and Oloomi, 2006).

In this study indicated in female more 13 years, the level of estradiol (30.60± 14.68 pg/ml) decline high significant ($p< 0.01$) compared with level in control group (13.83 ± 9.06 pg/ml) whereas the mean of testosterone levels were 1.22± 0.83 ng/ml in β-thalassemia patients and 3.71± 1.3ng/ml in control group more than 14 years. In β-thalassemia patients, mean level of testosterone were highly significant lower than in control ($p< 0.001$). In β-thalassemia patients low level estradiol (in females) and testosterone (in males) was noted 9/14(64.28%) and 7/9 (77.77%) respectively.

Dundar et al., (2007) was recorded the serum level of estradiol 19.4 ± 15.9 pg/ml significantly reduced ($p<0.001$) in β-thalassemia group compared with control group (72.1 ± 51.1 pg/ml) in puberty females, and in the males, serum levels of testosterone reduced in the β-thalassemia patients but not statistically significant.

Carmina *et al.* (2004) reported the serum level of testosterone(66 ± 123.66 ng/ml) in β-thalassemia patients significant decrease with healthy group(331.98 ± 173.76 ng/ml) in the male aged 14-18years . Other finding indicates lower serum levels of estradiol than controls of similar age. Additionally, in study by Hegazi et al., (2013) were compared the mean of the serum level of estradiol of patients with control group in the female aged between12.5-18 years. The results indicated significant ($p<0.05$) decreased the level of estradiol (22.91±17.41pg/ml) in the patients, when comparison with control group (108.17±107.45 pg/ml).

Immaturity is a profound problem of severe thalassemia. The association between hypogonadism and pituitary iron overload is well established, duo to pituitary iron overload begins in the first decade of life prior to the liver and cardiac iron deposition (Au *et al*., 2008 and Noetzli *et al*., 2012). Pituitary iron overload and iron-induced oxidative stress result in secondary hypogonadism in thalassemia patients (Roussou *et al*., 2013).

Vitamin D deficiency may appear early stage of life in thalassemia patients that contribute with low bone mass in thalassemia even before hypoparpthyroidism (Toumba and Skordis, 2010 and Saffari *et al*., 2012). Also, vitamin D stimulates intestinal calcium absorption. Notably, without vitamin D, only 10–15% of dietary calcium and about 60% of phosphorus are absorbed. So for, vitamin D sufficiency enhances calcium and phosphorus absorption by 30–40% and 80%, respectively (Lips *et al*., 2006 and Lappe *et al*.,2007).

The level of vitamin D statistically highly significant decline compared with control group. 94.74% of patients have vitamin D deficiently that compared with 78.94% of control group. the Vitamin D deficiency was observed in the almost cases; thalassemia patient and control. On the other hand, this could be to vitamin deficiency being a common finding the general population in Iraq.

Dandona et al., (1987) assessed the level of vitamin D in β-thalassemia significantly lower than control group.

Napoli et al., (2006) found mean 25-OH vitamin D was lower significantly in thalassemia patients (20.3 +/- 0.7 ng/ml) than healthy control person (25.2 +/- 1 ng/ml) in similar age whereas 10.1% had absolute deficiency of vitamin D. Other studies demonstrated that vitamin D deficiency was seen; 37.2% patients by Shamsiraz *et al*., (2003), in12% patients were deficient and 70% insufficient by Vogiatzi *et al*.,(2009), in 72.2% patients by Sultan *et al*.,(2016), while 100% patients by Soliman *et al*., (2008).

Fung et al. (2011) reported that in USA 43% of patients with thalassemia has vitamin D deficiency, which persisted despite daily low dose supplementation of 400-1,000 IU vitamin D. Other study by Fahim *et al*., (2013) reported the mean serum level of 25-OH vitamin D was significantly lower in children thalassemia patients than control group and 37 % had vitamin D deficiency and 54% had vitamin D insufficiency. In the recent reported 25-OH Vitamin D deficiency was been reported in 98% patients and 68% in control group. Mean difference of vitamin D level was statistically significant decreased in patients when compared with control group (Agrawal *et al*., 2016).

Vitamin D deficiency is general in patients with transfusional and iron overload, but the mechanism remains unclear. The first step of vitamin D metabolism, hydroxylation takes place in liver. Iron overload may interfere with this stage lead to malfunctioning hydroxylation of vitamin D rather than the dysfunction of endocrine tissues (Pirinççioğlu *et al*., 2011; Fahim *et al*., 2013 and Ezzat *et al*., 2015). Additionally, this deficiency may be attributed to malabsorption of vitamin D as well as insufficient dietary intake (Malik *et al*., 2009).Merchant et al., (2010) found vitamin D deficiency in 62% Indian thalassemia major children and suggested that vitamin D deficiency was nutritional deficiency and defective hydroxylation of vitamin D in liver due to hemochromatosis as all children had high serum ferritin levels.

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