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STUDY THE COMPLICATIONS OF ACCUMULATEDIRON IN THE TISSUE AND GLANDS IN PATIENTS WITH MAJOR β-THALASSEMIA

***[1]Basim M. Ali, A. L., [2]Furqan M. Auda, A. L., [3]Amar M. Ali and [4]Mansoor K. Abidali**

Department of Chemistry, College of Science, Al-Kufa University, Iraq

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ABSTRACT

In the present study, an attempt is carried out to estimate the level of sex hormone in thalassemia major patients where. The level of these compounds will indicate the risk of tissue damage caused by oxidative stress and iron overload in thalassemia patients. Thirty five Iraqi patients with major thalassemia were participated in the present study. Their age range was 2-12 years old. In other hand Thirty five apparently healthy children were selected as control group. Serum hormones (Leutinizing hormone (LH), Follicle-stimulating hormone (FSH), Testosterone, Estradiol (E2), Prolactine (PRL) were measured using ready for use ELISA kits supplied by Monobind®, Serum levels of iron was measured spectrophotometrically. The results showed significant increase ($p<0.05$) in iron level in patients with thalassemia as compared with their control group, in the other hand the results showed significant decrease ($p<0.05$) in all sex hormone(FSH&TEST.) of thalassemic patients in comparing with healthy control group except LH&PRO. Concentration, which on significantly changing ($p>0.05$) in those patients when comparing with the control group. It can be concluded that Iraqi thalassemic patients are at high risk for iron overload and iron-induced toxicities. These patients are prone to tissue injury caused by collection of iron in tissue.

INTRODUCTION

Beta-thalassemia represents a group of recessively inherited hemoglobin disorders first described by Cooley and Lee (Cooley & Lee, 1925) and characterized by reduced synthesis of β-globin chain. The homozygous state results in severe anemia, which needs regular blood transfusion. The combination of transfusion and chelation therapy has dramatically extended the life expectancy of thalassemic patients who can now survive into their fourth and fifth decades of life (Saka *et al*., 1995; Jensen *et al*., 1998). On the other hand, frequent blood transfusion in turn can lead to iron overload which may result in hypogonadism, diabetes mellitus, hypothyroidism, hypoparathyroidism and other endocrine abnormalities (Vullo *et al*., 1990). Several authors reported a high incidence of endocrine abnormalities in children, adolescents and young adults suffering from thalassemia major. However the incidence of the various Endocrinopathies changes among different series of the patients due to a mixture of reasons other than iron overloads (Bielinski *et al*., 2003). Thalassemia in Iraq is a real problem due mainly to the deficiency in the equipments and drugs during different periods of wars and lack of security. Out of 4168 patients recruited from the Public Health Laboratory in Basra, (southern Iraq). About 5% had beta-thalassemia trait and the carriers of major beta-globin disorders comprised 11.48% (Hassan *et al*., 2003). In Najaf Governorate (about 1.5 million people), till January 2013 there are 753 patients' files who are still treating in the "Thalassemia Unit" at AL-Zahra'a Teaching Hospital. Thalassemia major can result in severe complications and even death due to absence of hemoglobin a synthesis and the patients are more dependent on transfusion of blood (Hassan *et al*., 2003). Iron metabolism disorders are common in the human including both iron deficiency anemia and excessive iron storage. Iron is essential for oxidation-reduction catalysis and bioenergetics, but unless appropriately shielded, iron plays a key role in the formation of toxic oxygen radicals that can attack all biological molecules. Hence, specialized molecules for the acquisition, transport (transferrin), and storage (ferritin) of iron in a soluble nontoxic form have evolved. (Cunningham, 2008) In our country, there are more than 10,000 thalassemic patients. We have conducted this study to establish the pattern of endocrine function, bone

***Corresponding author: Basim M. Ali, A. L.,** Department of Chemistry, College of Science, Al-Kufa University, Iraq*

changes and nutritional status in Iraqi thalassemic patients. In the present study, the iron status was estimated in thalassemic patients and compared with control group. The second aim is to study the possible dependent of sex hormones levels (LH, FSH, PROL. & TEST.) on the concentration of iron status parameters in blood transfusion dependent thalassemic patients were examined.

MATERIALS AND METHODS

A-Patients: Thirty Five Iraqi patients with β-thalassemia major were participated in the present study. Their age range was 2-12 years old. These patients were registered as β-thalassemic major patients in "Thallassemia Unit" at "AL-Zahra'a Teaching Hospital" in Najaf city, (Iraq). The diagnosis was established by clinical symptoms, hematological, and hemoglobin high-pressure liquid chromatography (HPLC) analysis. Hemoglobin HPLC were done using (VARIANT^{TM} β-Thalassemia Short Program) HPLC instrument. All these patients were on blood transfusion as a part of their treatment regimen. Serum C-reactive protein (CRP) is negative in all samples (CRP<6mg/L). A normal C-reactive protein can be used to exclude elevated ferritin caused by acute phase reactions (Kennedy *et al*., 2004). The present study excluded the patients withapparent diabetes mellitus, infection and inflammation, heart diseases, and patients from non-Arabic ethnic groups.

B-Controls: Thirty Five apparently healthy children were selected as a control group. Their age ranges were comparable to that of patients. None of these subjects was anemic or has an noticeable systemic disease.

Measurements: Blood samples were collected from individuals in the morning in plain tubes and the serum separated by centrifugation after clotting. Serum levels of iron were estimated using Ferrozine colorimetric method (Artiss *et al*., 1981). The ferritin quantitative kit based on a solid phase enzyme-linked immunosorbent assay (ELISA) was supplied by Monobind® Inc. USA. The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtitre wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme horseradish peroxidase (HRP) conjugate solution. The LH quantitative kit based on a solid phase enzyme-linked immunosorbent assay (ELISA) was supplied by Monobind® Inc. USA. The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtitre wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme horseradish peroxidase (HRP) conjugate solution. FSH levels were measured using CellBiolabs®Follicle-stimulating hormone ELISA Kit. Briefly, bovine serum albumin (BSA) standards or protein samples are adsorbed onto a 96-well plate for two hrs at 37ºC. The FSH present in the sample or standard are derivatized with solid phase (DNPH) to DNP-hydrazon and probed with anti-DNP antibody, followed by an HRP conjugated secondary antibody. The protein carbonyl content in unknown sample is determined by comparing with a standard curve that is prepared from predetermined reduced and oxidized BSA standard (Reznick & Packer, 1994). CellBiolabs® PROL. Adduct ELISA Kit was used to measure PROL. level. It is an enzyme immunoassay for detection and quantization of PROL.-protein adducts.

The quantity of PROL. adduct in protein samples is determined by comparing its absorbance with that of a known PROL.-BSA standard curve. BSA standard or protein samples are adsorbed onto a 96-well plate for 2 hours at 37ºC. The PROL.-protein adducts present in the sample or standard are probed with an anti- PROL. antibody, followed by an HRP conjugated secondary antibody. The PROL. protein adducts in an unknown sample is determined by comparing with a standard curve that is prepared from predetermined PROL.-BSA standard (Yagi, 1998).The TEST. quantitative kit based on a solid phase enzyme-linked immunosorbent assay (ELISA) was supplied by Monobind® Inc. USA. The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtitre wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme horseradish peroxidase (HRP) conjugate solution.

RESULTS

Theresult in table(1) shows no significant change in serum level of (LH, PRL. &level of LH/FSH) in patients with thalassemic major as compared with their control groups. in addition the table shows a significant decrease in level of (FSH&TESTO.) ($p<0.05$) in patients with thalassemic major as compared with their control groups.In the other hand a significant increase in serum level of iron($p<0.05$) has been shown in this table in patients with major thalassemia as compared with their control groups. While in the same table shows significant increase in level of iron and ferritin in identical patients as compared with their control group.

Table 1.Serum of sex hormones and iron status in thalassemia and control group expressed as mean±standard deviation

Parameters	Control	Patients	Significance
FSH (mIU/ml)	5.9±3.32	3.45±2.94	0.047*
LH(mIU/ml)	2.4±2.33	2.5±3.52	N.S
LH/FSH	0.406±0.08	0.769±0. 2	N.S
Testo.(ng/ml)	6.1±3.12	2.6±2.3	0.021*
PRL (ng/ml)	4.39±5.47	3.95±4.17	N.S
IRON. µg/dl	67.8±42.5	116±43	0.001**
FERR.ng/ml	63±34	129±57	0.038*

Note:= significant , ** = high significant*

The statistical analysis in table (2) demonstrated that there is no significant difference between male and female patients regarding their iron, LH, PRL, LH/FSH, FSH, TESTO & ferritin levels.

Table 2. Effect of sex on serum sex hormones and iron status concentration in thalassemia patients

Parameters	Female	Male	Significance
FSH (mIU/ml)	3.05±2.92	3.45±2.94	N.S
LH(mIU/ml)	2.4±2.33	2.5±2.32	N.S
LH/FSH	0.506±0.08	0.649±0. 2	N.S
Testo.(ng/ml)	3.1±2.12	2.6±2.2	N.S
PRL (ng/ml)	4.39±3.47	3.95±3.17	N.S
IRON. µg/dl	107.8±32.5	116±43	N.S
FERR.ng/ml	133±34	129±57	N.S

The statistical analysis in table (2) demonstrated that there is no significant difference between male and female patients regarding their iron, LH, PRL, LH/FSH ratio, FSH, TESTO & ferritin levels.

Table 3.The correlation for both iron and ferritin with (LH, FSH, TESTO., PRL. & LH/FSH)

parameter	Correlation with iron(r)	P value	parameter	Correlation with ferritin(r)	P value
LH	-0.307	0.422	LH	-0.053	0.893
FSH	-0.279	0.467	FSH	-0.276	0.472
LH/FSH	0.725 *	0.027	LH/FSH	0.861*	0.003
Testo.	0.094	0.809	Testo.	0.083	0.831
PRL	0.350	0.356	PRL	0.197	0.611
Ferr.	0.849 *	0.004	iron	0.849 *	0.004

Note:= significant*

The result in table (3) demonstrated no relationship between all parameters of sex hormone versus iron and ferritin levels in thalassemia patients except a positive correlation in ratio of LH/FSH with iron and ferritin levels in thalassemia patients, in addition a positive relation between iron and ferritin levels in thalassemia patients.

DISCUSSION

The results of iron indices in thalassemic patients indicated a state of iron overload. In iron overload state, the iron which is initially stored as ferritin, is deposited in organs as haemosiderin and this is toxic to tissue, probably at least partially by inducing oxidative stress (Lekawanvijit & Chattipakorn, 2009). Most humans avoid iron overload solely by regulating iron absorption. Those who cannot regulate absorption well enough get complaints of iron overload. In these diseases, the toxicity of iron starts crushing the body's ability to bind and store it. Patients with thalassemia major accumulate body iron over time as a consequence of continuous red blood cell transfusions which cause hepatic, endocrine, and cardiac complications (Lekawanvijit & Chattipakorn, 2009); (Galanello *et al*., 2010). All these studies are associated with the current study.Impaired puberty, which occurred in about69 % of our patients, was the most common endocrine defect. The occurrence of other endocrinopathies was much lower: 23 % hypogonadism. Diminished puberty seems to be more prevalent in our study compared to study of Hypogonadism in our study was considerably lower than other studies. In a longitudinal study, prevalence of hypogonadism has been reported to be as much as 63% in girls and 58% in boys (Kwan *et al*., 1995).

It is unclear whether endocrinopathies in β-thalassemia major is connected to genetic influences (Kwan *et al*., 1995). It seems that our endocrine disorder patients were of younger ages at the time of diagnosis in comparison with other studies (Chern, 2001). It is important to note that even in the studies in which the prevalence of overt hypogonadism as a complication of thalassemia major is relatively lowMilder forms of thyroid dysfunction are much more common (Magro *et al*., 1990); (Depaz *et al*., 1985); (Landau *et al*., 1993), though again there are wide variations in different reports. These discrepancies cannot be attributed to differences in patients' ages, but rather to difference treatment protocols, including differing transfusion rates and chelation therapies (Phenekos *et al*., 1984); (Tanner & Whitehouse, 1976); (De Sanctis & Wonke, 1998). Short stature seemed to be more prevalent among our patients compared to other studies (Flynn *et al*., 1976). Our growth assessments did not show any difference of short stature prevalence between prepubertal and pubertal patients, in contrast to the results of Pignatti *et al* who claimed growth abnormalities to be more prevalent in pubertal patients (Pignatti *et al*., 1985). High prevalence of endocrine abnormalities was reported by several authors (Canale *et al*., 1974). They demonstrated that these abnormalities were related to iron overload. The histological studies of different endocrine glands supported this hypothesis (Costin *et al*., 1979). We found significant difference in mean serum ferritin level between thalassemic patients with primary amenorrhea, irregular mense, hypogonadism and those without endocrinopathies. These findings yield the importance of iron overload in development of endocrine disorders. In contrast, there are some other reports which have suggested no relation between the level of ferritin and some other endocrinopathies (Zervas *et al*., 2002). It has been suggested that the prognosis for survival is excellent for thalassemic patients with serum ferritin concentration below 2500 µg/l Olivieri *et al*., 1994). We found a considerable sum of endocrinopathies in our population. Taking into account that their ferritin levels are not of high amounts, it is possible, therefore, that there are other factors responsible for organ damage. Among these factors; liver damage due to viral infections, increased activity of the iron dependent protocollagenproline hydroxylase enzyme, chronic anemia and individual susceptibility to damage from iron overload have been previously pointed out.The changes in serum ferritin concentration during development from birth to old age reflect changes in the amounts of iron stored in tissues. There is a good correlation between serum ferritin concentration and storage iron mobilized as a result of phlebotomy. This suggests a close relationship between the total amount of stored iron and the serum ferritin concentration in normal individuals (Worwood, 2008). Ferritin synthesis is induced by administering iron.

Conclusion

High prevalence of endocrine and metabolic complications among our thalassemics signifies the importance of therapeutic interventions. The differences of these abnormalities in different series of patients may be due to variations in treatment protocols or different susceptibilities and demand more detailed studies.

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