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EVALUATION OF VITAMIN D AND OSTEOPOROSIS STATUS PARAMETERS IN BETA -THALASSEMIA MAJOR PATIENTS IN HILLA PROVINCE

Radhwan M. Hussein[1]*, Fatima Adnan Alzubaidi[2] and Zainab Sajid Mohammed[3]

[1]College of Pharmacy, University of Ahl AL-Bait, Iraq.
[2]Department of Pharmacology and Toxicology, College of Pharmacy, University of Babylon, Iraq.
[3]College of Pharmacy, University of Ahl AL-Bait, Iraq.
*e-mail : radwan.asal@yahoo.com

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ABSTRACT : Beta thalassemia is a genetic disorder, characterized by the complete absence or decrease synthesis of β globin chains. Regular blood transfusion is the mainstay of treatment of severe β thalassemia. Iron overload is the result of repeated blood transfusion with many complications including endocrinopathies, behavioral and neurotic problemsetc. To investigate the levels of Vitamin D, calcium, phosphate, parathyroid hormone, ferritin and enzymes of the liver (alanine aminotransferase, ALT and aspartate aminotransferase, AST), in the sera, of thalassemic and normal persons and to correlate these parameters with osteoporosis prevalence. This study was case control study in which ninety person were involved and subdivided by three groups: control group (Thirty healthy individual), Thalassemia group (Thirty thalassemic patients without osteoporosis) and Thalassemia group with osteoporosis (Thirty thalassemic patients with osteoporosis). Blood samples were collected and serum used to evaluate the mentioned parameters. Ca, vit. D and parathyroid hormone level decreased significantly in thalassemic and Thalassemic osteoporosis patients, while Phosphate, ferritin, ALT and AST increased significantly when compared to control group. Iron overload is one of complications in patients suffering from beta thalassemia major also required Ca and vitamin D supplements with periodic checking of serumCa, vit D, PTh, ferritin and liver function.

Key words : β-thalassemia, osteoporosis, PTH, calicum.

INTRODUCTION

Thalassemia are inherited group of hematological illnesses, characterized by a reduction in single or moreglobin chains synthesis that leads to disturbance in globin chain synthesis. Incorrect hemoglobin production makes red blood cells highly vulnerable to destruction, that cause hemolysis, impair erythropoiesis and lead to anemia (Rund *et al*, 2005). There are two major types of thalassemia : Alpha and Beta depending on which globin chain portion is produced in low amounts (Herbert *et al*, 2009).

Beta thalassemia occurs when there is a decrease or absent production of the beta globin chains, that produce very high alpha chains amount. Beta globin production is managed by one gene on every chromosome (Agouzal *et al*, 2010). Beta thalassemia happened by one of the 200 or more point mutations also by deletions of both genes but this is rare. Beta globin chain synthesis can ranged from subnormal to absolute absent, causing different degrees of increase in alpha globin compared beta globin chain synthesis. Beta thalassemia trait (minor) occurred when there is a single gene defect, which is without symptoms and lead to microcytosis, with mild anemia, when the production from two genes highly decreased or absent then patient has what called beta thalassemia major, and called also the Cooley anemia. Beta thalassemia major patients are mostly have no symptom at birth because HbF presence, by six months of age symptoms begin to develop. If the production of beta chains decreased with lower severity and the patient called has beta thalassemia intermedia, these patients show symptoms that are lower severity and transfusions for lifelong to survive beyond twenty year of age do not required (Renzo and Raffaella, 2010).

β-thalassemia complication includes: iron over load, infections, bone marrow deformities with abnormally bone structured, particularly in the face and skull, slow growth rate, heart problems and enlarged spleen. Patients with β-thalassemia can show iron overload in their bodies, either by the disease itself or by repeated transfusions of blood. Excessive iron can lead to harmful effect to many parts such as, liver, heart, also the endocrine system, that

includes glands responsible for production of hormones that regulate pathways throughout all the body (Cianciulli, 2008), the majority of worsening of excess iron in the children include growth defects also failure in the sexual maturation (Borgna-Pignattiand Galanello, 2004), β-thalassemia major become fetal disease if the patients are treated inadequately (Ismail *et al*, 2006).

Thalassemia major, in an infantal most suspected before two years old with mild jaundice, very severe microcytic anemia, also hepatosplenomegaly (Kirti *et al*, 2013).

Beta thalassemia major patients need blood transfusions periodicly and lifelong, in order to save a hemoglobin value above 9.5 g/dL (95 g/L) and maintain normal growth. The need for transfusions of the blood may begin early as 6 months after birth. The patients with transfusion-dependent mostly have iron overload due to there is no physiologic process to eliminate over iron from multi transfusions, subsequently, they need treatment by chelator for iron begin between 5 years old and 8 years old, Deferoxamine (Desferal^{R}), by subcutaneously rout or intravenously, is the management of choice for this case. Transplantation of bone marrow during childhood is the mainly curative tratment for beta thalassemia major (Thuret, 2000; Dharmesh *et al*, 2017).

MATERIALS AND METHODS

This study was case control study in which ninety subjects were included and subdivided for three groups during the period from February to September 2018, in the Thalassemia center in Hilla city at, the Pediatric and Maternity Teaching Hospital in which they were grouped into three groups; the first one: control group (Thirty healthy individual), second group: Thalassemia group (Thirty thalassemic patients without osteoporosis) and third group: Thalassemia group with osteoporosis (Thirty thalassemic patients with osteoporosis). Our study was approved by Institute Review Board in Ahl-AL Bait University, College of Pharmacy. Each participant or his parents gave a written consent showing his agreement for the participation in this study.

Samples were collected in the morning, five milliliters of venous blood was taken from each subject using disposable syringes in the sitting position, the blood evacuated slowly into plain disposable test tubes without anticoagulant and left for ten minutes at 37^{0}C in plane tube for clotting, then Centrifuged at 1000 xg, for 10 min in order to obtain the serum, the obtained serum subdivided into two parts in order to measure the experimental parameters as explained blow, the first part used to measure total calcium (Spinreact company), Phosphorus (Linear Chemicals company) and alanine aminotransferase (ALT) and aspartate aminotransferase (AST), (Randox company) by colorimetric method and by procedures provided by manufacturing company (Retiman and Frankel, 1957).

The second part of the sera that obtained stored in two eppendorf tubes at -80^{0}C until all the collection of samples completed and then we measure theVitamin D (MyBiosource company), parathyroid hormone (DRG Company) and Ferritin (abcam company) by using Enzyme-Linked Immunosorbent Assay (ELISA), method depending on the procedure provided with the kits from the manufacturing company (Alciato *et al*, 2008).

Statistical analysis

By using program of computer (SPSS 24 IBM, Armonk, USA) Statistical Package for Social Science. The results were expressed by a mean of: mean ± standard error of mean (SEM). The difference was considered statistically significant when P-value of <0.05. Results were compared by (ANOVA) and the least significant differences (LSD) were calculated for all the differences in mean of the variables between control and thalassemia group and thalassemia with osteoporosis group (Armitage *et al*, 2002).

RESULTS AND DISCUSSION

The results of age, vitamin D, calcium, phosphate, ferritin, PTH, ALT and AST for control, thalassemia and thalassemic osteoporosis groups are listed in Table 1.

In the current study the mean ± SEM of age for control ,thalassemia and thalassemic osteoporosis groups were :23.87±0.41years, 25.00±0.86 years and 24.6 ±0.62 years, respectively. There were no significant variance (P<0.05) in the mean of age among all groups.

The mean ± SEM of serum ferritin for control, thalassemia and thalassemic osteoporosis groups were 163.92±4.0 (ng/ml), 575.8±0.12 (ng/ml), and 598.14 ±11.6 (ng/ml), respectively. There were: very high statistical sig.elevation (P>0.001), in the mean of serum ferritin level in thalassemia group also thalassemic osteoporosis groups in comparison with control group, while there were: insig. difference (P<0.05) in thalassemic osteoporosis group in comparison with, thalassemia group. This result is in harmony with the study of Meenu *et al*, this is due to that such patients are treated with blood transfusion that results in iron overload (Herbert *et al*, 2009; Khaled *et al*, 2013; Adlette *et al*, 2015).

The mean ± SEM of serum Parathyroid hormone for control, thalassemia and thalassemic osteoporosis groups

Table 1 : The mean ± SEM of age, vitamin D, calcium, phosphate, ferritin, PTH, ALT and AST for control, thalassemia and thalassemic osteoporosis groups.

Number	Control Group 30	ThalassemiaGroup 30	Thalassemic osteoporosis group 30
Mean ± SEM of Age (Year)	23.87±0.41	25.00 ±0.86^{aNs}	24.6 ±0.62^{aNs,bNs}
Mean ± SEM of Calcium (mg/dl)	9.99±0.21	7.7±0.37$^{a▼**}$	6.36 ±0.48 $^{a▼***, b▼*}$
Mean ± SEM of Phosphate (mg/dl)	3.44±0.11	4.15 ±0.33 $^{a▲*}$	4.79 ±0.14 $^{a▲**, ▲b*}$
Mean ± SEM of Vitamine D (ng/ml)	23.56±2.31	15.27 ±0.56$^{a▼***}$	6.36 ±0.65$^{a▼***,b▼***}$
Mean ± SEM of Ferritin (ng/ml)	163.92±4.0	575.8±0.12$^{a▲***}$	598.14 ± 11.6$^{a▲***,b\ Ns}$
Mean ± SEM of Parathyroid hormone (ng/ml)	42.38±0.81	37.01±0.78 $^{a▼**}$	36.00±0.81 $^{a▼***, bNs}$
Mean ± SEM for ALT (IU/l)	30.19±0.51	39.45 ±0.5 $^{a▲***}$	40.85 ±0.43 $^{a▲***,b\ Ns}$
Mean ± SEM for AST (IU/l)	28.32±0.56	37.9 ±1.48 $^{a▲***}$	39.6 ±0.88 $^{a▲***▲b*}$

a= ANOVA test between Thalassemia , Thalassemic osteoporosis groups and control group in which NS= insignificant difference; $^{▼**}$= high significant decrease (P<0.01); $^{▼***}$= very high, significantdrop (P<0.001); $^{▲***}$=very high; significant elevation (P<0.001); $^{▲*}$= significant, elevation (P<0.05); $^{▲**}$ = high, significant elevation (P<0.01).

b = analysis of variance, test between Thalassemia with Thalassemic osteoporosis groups: NS = insignificant difference; $^{▲*}$= significant elevation (P<0.05); $^{▼*}$= significant,drop (P<0.01); $^{▼***}$= very high, significant drop (P<0.001).

were: 42.38 ± 0.81 (ng/ml), 37.01 ±0.78 (ng/ml) with 36.00±0.81 (ng/ml), respectively. There were: very high statistically, significant decrease (P<0.001), in the mean of serum Parathyroid hormone level in thalassemia group and thalassemic osteoporosis group in comparison, with control group, while there were, insignificant difference (P<0.05), in thalassemic osteoporosis group in comparison, with thalassemia group. The decrease in serum level of Parathyroid hormone is due to iron overload in β-thalassemia patients (Alain and Marengo-Rowe, 2007), there are different mechanisms that may explain the possibility of incidence of glandular damage by iron overload, one of these mechanisms is the formation of free radical and lipid peroxidation that lead to damage in different sites including mitochondrial membrane; sarcolemmal membrane; and lysosomal membrane (Bandebuche *et al*, 2013).

The mean ± SEM of serum calcium for control, thalassemia and thalassemic osteoporosis groups were 9.99±0.21(mg/dl), 7.7±0.37 (mg/dl) and 6.36 ±0.48 (mg/dl), respectively. There were high statistically significant decrease (P<0.01), in the mean of serum calcium level in thalassemia group as compared, with control group, while there were very highstatistically significant decrease (P<0.001), in thalassemic osteoporosis group as compared with control group and statistically significant decrease (P<0.05), as compared with thalassemia group. Our results are in line with that mentioned by Napoli *et al* (2000), which is because hypoparathyroidism (Bandebuche *et al*, 2013).

Low para-thyroid hormone levels cause excessive urine calcium loss, decrease bone remodeling and lowercalcium absorption by intestine (Cooper and Gittoes, 2008).

The mean ± SEM of serum phosphate for control, thalassemia and thalassemic osteoporosis groups were 3.44±0.11 (mg/dl), 4.15 ±0.33 (mg/dl), with 4.79 ±0.14 (mg/dl), respectively. There were statistical significant increase (P<0.05), in the mean of serum phosphate level in thalassemia group in comparison with control group, while there were high statistical significant increase (Pa<0.01), in thalassemic osteoporosis group as compared with control,andstatistical significant elevation (P<0.05), when compared with thalassemia group, this result was similar to a previous study conducted by Shah (2015), which is also due to hypoparathyroidisim. PTH reduces the kidney reabsorption of phosphate by the proximal tubule, which means more urinaryphosphate excretion , therefore, hypoparathyroidism is associated with elevated serum phosphate level (Shah, 2015).

The mean ± SEM of serum vitamin D for control, thalassemia and thalassemic osteoporosis groups were 23.56±2.31 (ng/ml); 15.27 ±0.56 (ng/ml); and 6.36 ±0.65 (ng/ml); respectively. There were very high statistical significant drop (P<0.001), in the mean of serum Vitamin D level in thalassemia group and thalassemic osteoporosis group in comparison with control group, while there were very high statistical significant decrease, (P<0.001), in thalassemic osteoporosis group when compared with thalassemia group. This result is in consistent with the findings of Napoli *et al* (2000), the decrease in vitamin D is due to decrease in hydroxylation to 1, 25-dihydroxy vitamin D3 that takes place in the kidney, which is controlled by parathyroid gland (Ashraf *et al*, 2013).

The mean ± SEM of serum ALT for control, thalassemia and thalassemic osteoporosis groups were 30.19±0.51 (IU/l), 39.45 ±0.5 (IU/l) and 40.85±0.43 (IU/l), respectively. There were very high statistical significant elevation (P<0.001), in the mean of ALT level in thalassemia group also thalassemic osteoporosis groups in comparison with the control group, while there were insignificant difference (P<0.05), in thalassemic osteoporosis group in comparisonbythe thalassemia group.

Mean ± SEM of serum AST for control, thalassemia and thalassemic osteoporosis groups were 28.32±0.56 (IU/l), 37.9±1.48 (IU/l) and 39.6±0.88 (IU/l), respectively. There were: very high statistical significant increase (P<0.001), in the mean of serum AST level in thalassemia group and thalassemic osteoporosis groups,in comparison by the control group, while there were: statistical significant increase (P<0.05), in the thalassemic osteoporosis group as compared with the thalassemia group.

Our results are in line with that reported by Ashraf *et al* (2014). Liver is the major site of storefor iron,also the single site of transferrin and ferritin production. Free ferrous ironin normal condition is protein-bound in the liver and highly toxic. Free (Unbound), iron trigger the synthesis of free radicals;thatplay a main role in the peroxidation of lipids, also liver toxicity. The peroxidation of lipids perhaps the initial event that causing hepatocellular defects secondary to the iron overload (Li *et al*, 2002; Ashraf *et al*, 2014; Zahraa *et al*, 2018).

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