Changes in serum iron, total iron binding capacity, calcium and phosphorus concentrations in children with beta thalassemia major in Babylon Governorate

Muhammad Obaid Al-muhammadi, M.Sc., PhD. Physiology
University of Babylon ,College of Medicine

الخلاصة

صممت هذه الدراسة لمعرفة تأثير مرض فقر الدم البحري البائي الكبير على بعض المتغيرات الكيميوحيوية لدى الأطفال المصابين بذلك المرض من الذكور والإناث في بابل. شملت هذه الدراسة فحص ٥٠ (٢٥ ذكر و ٢٥ أنثى) طفل من المصابين والذي كان معدل أعمارهم ٩ ± ٥ سنة. جميع الأطفال المرضى تم عليهم اجرائات نقل الدم الدوري وكذلك إعطائهم مادة الدسفيرال. وشملت مجموعة السيطرة ٢٦(١٣ ذكر و ١٣ أنثى) طفل من الأصحاء ومن كلا الجنسين في هذه الدراسة.

تميزت التغيرات الكيميوحيوية لدى الأطفال المصابين بمرض فقر الدم البحري البائي الكبير بارتفاع تركيز الحديد في مصل الدم (serum iron) (للذكور والإناث) بصوره معنوية ($p<0.01$) مقارنة بالأطفال الأصحاء. بينما لوحظ انخفاض معنوي ($p<0.01$) في قيمة سعة الحديد الكلية في مصل الدم (TIBC) عند المقارنة بمجموعة السيطرة ولكلا الجنسين. إما فيما يتعلق بنتائج تركيز الكالسيوم في مصل الدم فقد أظهرت انخفاضا معنويا ($p<0.05$) للأطفال المرضى من الذكور والإناث. في حين سجلت أقيام تركيز الفسفور في مصل الدم (للذكور والإناث) زيادة معنوية ($p<0.05$) مقارنة بمجموعة السيطرة.

إن المتغيرات الحاصلة في نتائج هذه الدراسة يمكن إن تعزى بصورة رئيسية إلى الدور السام للحديد و المترسب في معظم أنسجة أعضاء الجسم كالكبد، الكلية، القلب، الغدد والعظام والناتج عن عملية نقل الدم المستمر.

Abstract

This study was designated to investigate the changes of some biochemical parameters in children affected with beta thalassemia major in Babylon. Fifty transfusion dependent patients beta thalassemia major (25 males and 25 females) included in present study. The mean of their ages was 9 ± 5 years ,those patients undergo periodical blood transfusion and desferroxamine as a chelating agent. Twenty six healthy controls (13 males and 13 females) of matched age and gender were also included in this study. Beta thalassemia major patients showed a significant increase ($p<0.01$) in values of serum iron (in both males and females) incomparsion with control. While the values of total iron binding capacity recorded a significant decrease ($p<0.01$) compared with control. As well as values of serum calcium pointed out a significant decrease ($p<0.05$) in patients with beta thalassemia, whereas the results of serum phosphorus showed a significant increase ($p<0.05$) in both males and females with beta thalassemia major as compared with healthy subjects. The results of this study may be attributed to toxic roles of iron over load on organs such as liver, kidney, heart, glands and bones.

Introduction

Thalassemias are one of the most common genetic disorder on a worlds basis. It represent the commonest cause of chronic hemolytic anemia in middle east. Beta thalassemia major is a hemolytic state of an inherited defect in beta-globin chain synthesis [(1)].Beta thalassemia major was first described by a Detroit pediatrician, Thomas Cooley ,in 1925[(2)]. Individuals with beta thalassemia major usually present within the first two years of life with sever anemia ,requiring regular red blood cells (RBCs) transfusions [(3)]. In beta thalassemia major, impaired biosynthesis of the beta-globin leads to accumulation of unpaired alpha-globin chain, shortened red cell life span

and iron overload cause functional and physiological abnormalities in various organ system[4]. Present transfusion regimens protocols have increased the life expectancy with beta thalassemia major but caused a progressive iron overload[5]. As a results of iron overload, those patients develop liver, heart endocrine abnormalities[6; 7]. Patients with beta thalassemia major go through several complications such as the transfusion-related infections like hepatitis B, C and I virus (HBV , HCV , and HIV) [8] . Iron overload complications are also noticed that includes endocrinopathies , heart and liver diseases in addition to chelation therapy complication [9].Beta thalassemia major is associated with characteristic bone deformities that are considered the result of continuous massive ineffective erythropoiesis These abnormalities resolve with regular transfusions starting early in life .Despite current management ,there is growing awareness that adults with beta thalassemia major. Frequently manifest low bone mass and complain of bone pain [10].Clinical management of beta thalassemia major consist in regular long-life RBCs therapy to remove iron introduced in excess with transfusions. At present, the only definitive cure is bone marrow transplantation[11;12].Prenatal diagnosis of thalassemia is possible by analyzing DNA obtained via chorionic villi sampling at 8-10 weeks of gestation or by amniocentesis at 14-20 weeks of gestation .Since genetic therapy strategies are currently in the early stages of development [13].Patients with beta thalassemia major have inevitably suffered from complications of disease, due to iron overload. This major causes of death in this group of patients are congestive heart failure and fatal cardiac tachyarrhythmias leading to sudden cardiac death[14;15].

The aim of the study:-

This study aims to estimate some biochemical changes to beta thalassemia major patients because this will help the medical staff for proper management with less morbidity and mortality. So this study is designed to determine the followings:-

1:-Serum iron and serum total iron binding capacity.

2:-Serum calcium and serum phosphorus.

Patients and Methods

The patients:-

The present study included 50 patients (25 males and 25 females) affected with beta thalassemia major ,that undergo periodical blood transfusion and desferoxamine as chelating a gent . This study was carried out over 4 months in thalassemia center in Babylon .The tasted group aged from 4 – 14 years (9 ± 5 years).The diagnosis of beta thalassemia major were made based on the clinical ,hematological and hemoglobin electrophoresis profiles which is indicative signs of the presence of beta thalassemia major. Twenty six healthy controls(13 males and 13 females) of matched age and gender were also included in this study .All patients were transfusion dependent at a rate one to two times monthly. Also ,all of them are treated with desferal.

The methods:-

Blood collection:-

The collection of blood was performed in the thalassemia center in Babylon. Collection was always performed at 9 a.m. by using venipuncture needles. The plain tubes, for blood to be used for preparing sera for subsequent biochemical tests. The blood is allowed to clot for 45 minutes, the clot shrinks and serum can be obtained by centrifugation and precautions were taken to avoid hemolysis. The serum samples were

isolated in sterile test tubes using micropipette with sterile disposable tips. Each sample was labeled and given a serial number together with the patient name, the serum samples were frozen at 20°C for biochemical analysis [16].

Biochemical studies:-

1-Determination of serum iron:-

The iron dissociated from transferrin-iron complex by a solution of guanidine acetate and reduced by ascorbic acid reacts with ferrozine to give a pink complex (according to procedure recommended by the serum iron from Biomaghreb company) [17].

2-Determination of serum total iron binding capacity (TIBC):-

An excess of iron is added to the serum to saturate the transferrin .The unbound iron is precipitated with basic magnesium carbonate (according to procedure recommended by the serum total iron binding capacity from Biomaghreb company) [18].

3-Determination of serum calcium:-

Calcium in the sample, reacts with O-cresolphtaleine at alkaline pH. The colored complex formed is proportional to the amount of calcium concentration in the sample. The intensity of the color was measured photometrically by using spectrophotometer at 570 nm wave length (according to procedure recommended by the serum calcium from Human company,Germany) [19 ; 20].

4-Determination of serum phosphorus:-

Inorganic phosphorus reacts with molybdic acid forming a phosphomolybdic complex . Its subsequent reduction in alkaline medium originates a blue molybdenum. (According to phosphorus kit from spinreact) [21].

Statistical analysis:-

All values were expressed as means ± standard error (SE).The data were analyzed by using computerized SPSS program. Independent t-test was used to estimate differences between groups .The differences were considered significant when the probability (P) was less than 0.05 ($P<0.05$) and highly significant when the probability (P) was less than 0.01 ($P<0.01$) [22].

The results:-

1- Serum iron:-

The results of serum iron of beta thalassemia major and control are presented in figure 1. The mean and stander error of serum iron for males and females of beta thalassemia major patients were : 180 ± 0.432; 166.5± 0.52 microgram/deciliter (Mg/dL), respectively) and were significantly ($P<0.01$) higher than control males and females (115.123 ±0.721 ; 113.5 ± 0.41 Mg /dL, respectively).

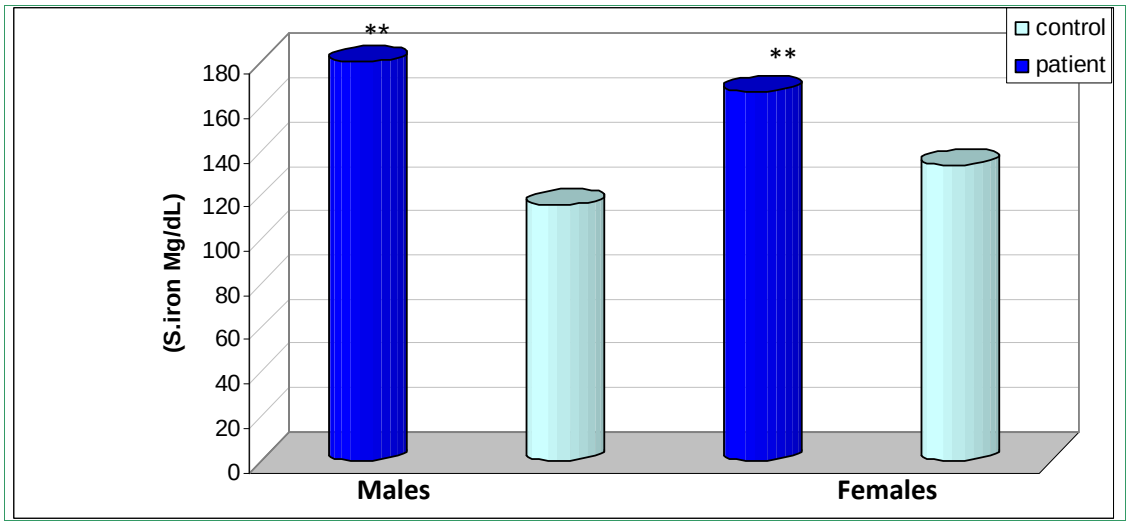

Figure 1 :Shows the means of serum iron values in children (male and female) with beta thalassemia major .
-**p <0.01

2-Serum total iron binding capacity (TIBC):-

Figure 2 shows the results of serum total iron binding capacity of beta thalassemia major and control .The mean and stander error of serum TIBC for males and females of beta thalassemia major patients were: 210 ± 0.823; 195.4 ± 0.33 microgram/deciliter (Mg/dL), respectively) and were significantly (P<0.01) lower than control males and females (250.4 ±0.52 ; 244.5 ± 0.62 Mg /dL, respectively).

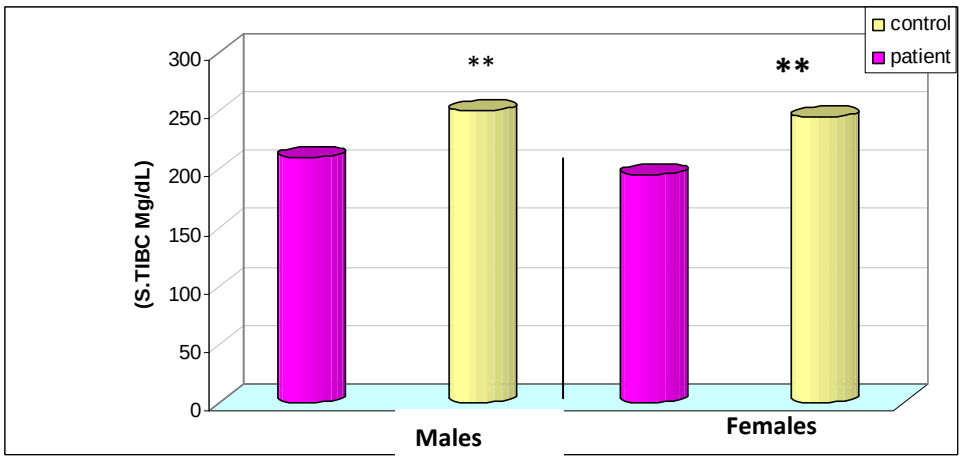

Figure 2 :Shows the means of serum TIBC values in children (male and female) with beta thalassemia major.

-p <0.01**

3-Serum calcium:-

The values of serum calcium of beta thalassemia major and control are illustrated in figure 3. The mean and stander error of serum calcium for males and females of beta thalassemia major patients were: 8.9 ± 0.21; 9.3± 0.312 milligram/deciliter (mg/dL),

respectively) and were significantly (P<0.05) lower than control males and females (11.81 ±0.21 ; 12.0 ± 0.112 mg /dL, respectively).

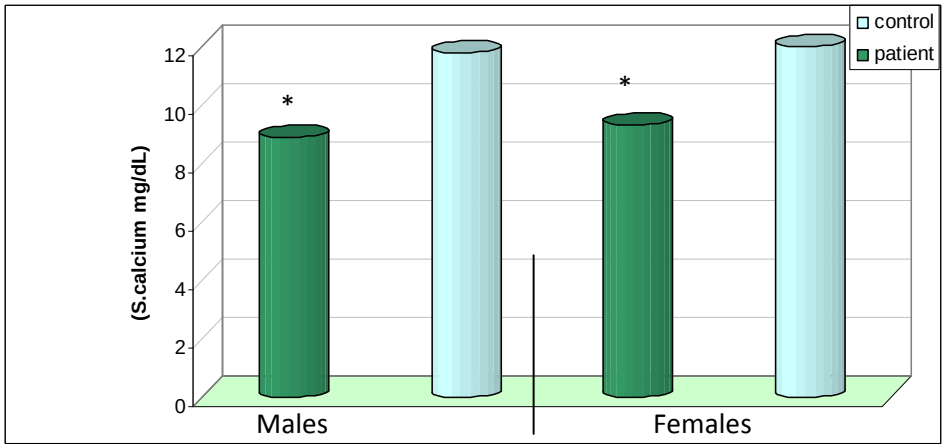

Figure 3: Shows the means of serum calcium values in children (male and female) with beta thalassemia major.

-*p <0.05

4-Serum phosphorus:-

Results of serum phosphorus of beta thalassemia major and control are presented in figure 4.The mean and stander error of serum phosphorus for males and females of beta thalassemia major patients were: 5.212 ± 0.311; 4.9 ± 0.30 milligram /deciliter (mg/dL), respectively) and were significantly (P<0.05) higher than control males and females (3.91 ±0.102 ; 3.5 ± 0.103 mg/dL, respectively).

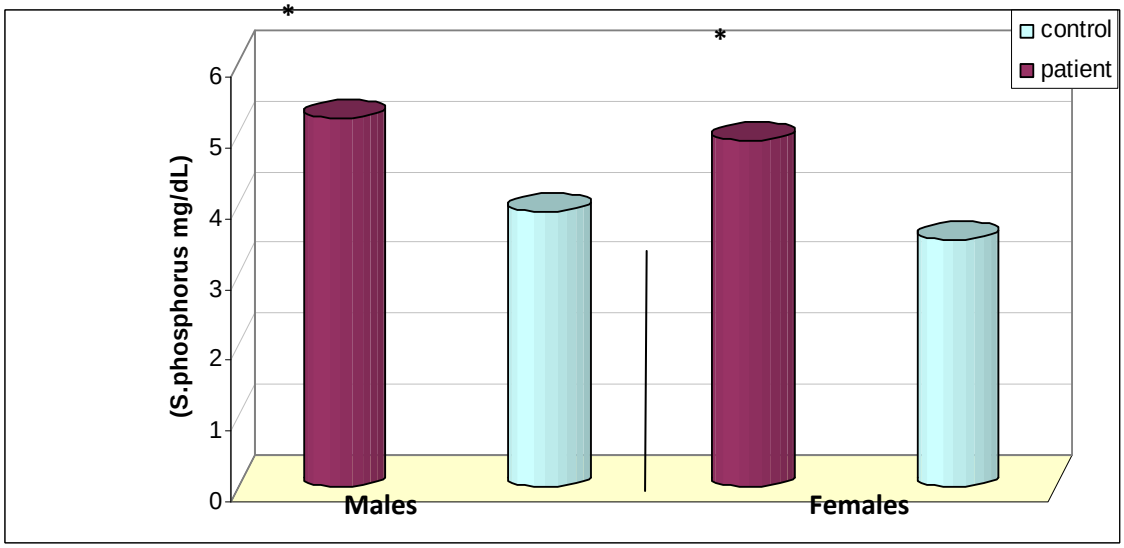

Figure 4 :Shows the means of serum phosphorus values in children (male and female) with beta thalassemia major.

-*p <0.05

Discussion

The present study investigated the relationship between iron overload and some biochemical parameters in blood of children affected with beta thalassemis major. It was found that the majority of the participants had significant increase of serum iron with significant decrease of total iron binding capacity(fig.1,2).These results are in agreement with other studies [23; 24; 25]. As well as ,Ghone *et al.*, [26], showed significant increase in serum iron whereas significant decrease in serum TIBC in beta thalassemia major. The patients with beta thalassemia major have sever anemia due to ineffective erythropoiesis which is primary reason for iron overload and blood transfusion is secondary to it. Thus, increased iron may increase the potential of oxidative injury to erythrocyte and cell organelles [27; 28]. Moreover, because of regular blood transfusion ,all beta thalassemia major patients had abnormally very high levels of total serum iron ,indicating that these patients have iron overload, probably due to multiple blood transfusion ,increased dietary iron absorption or in adequate chelating therapy with desferal [29]. Patients with beta thalassemia major undergo from massive distraction of abnormal red blood cells in reticuloendothelial system, and those patients become undergo from sever anemia [30].

These changes were associated with significant decrease in serum calcium and significant increase in serum phosphorus (fig.3,4).

These results of serum calcium and phosphorus were agreed with Rioja *et al.*, [31]; Rasheed and Ahmed [32]. A low calcium level was probably caused by a combination of hypoparathyroidism and osteomalacia evidenced by elevated bone alkaline phosphatase presumably resulting from deficient calcium intake [33]. From physiological point view, it appear clearly that high levels of iron have toxic roles on vital organs and disturbs these organs to perform their own normal functions [34]. Lack of physiological mechanism in beta thalassemia major to eliminate the excessive iron causes its deposition in tissues ,when the iron – binding capacity of iron binding proteins such as transferring and ferritin is exceeded, non-transferrin bound iron can generate harmful free radicals and cause tissue and multi-organ damage[4; 35]. Hormones of parathyroid gland especially parathyroid and calcitonin hormones regulate normal levels of calcium and phosphorus in blood, and this gland becomes insufficient to produce these hormones because precipitation of iron in tissues of this gland [36].

It was documented that the function of osteoblast is reduced, which is thought to be the major cause of osteopenia and osteoporosis in beta thalassemia major[37]. Osteoporosis is the most prevalent bone complication in beta thalassemia patients despite regular transfusion and iron chelation therapy. The 25-hydroxyvitamin D3 and bone mineral density were significantly decreased among patients with beta thalassemia [38]. Moreover, other studies are in agreement with the finding of our study on calcium homeostasis (Fig.3)[39];[40].

Conclusion

Regular (chronic) blood transfusions in beta thalassemia major lead to iron overload as well as in adequate chelating therapy considered the corner stone for the changing the our results of biochemical parameters (serum iron; TIBC ;calcium and phosphorus).

References

(1)-Khalifa A. S.; Baffico M.; Heshmat N. M.; Romco G. and Rody M. S.(1995). Relationship between the hematological phenotype and type of beta thalassemic mutation in Egyption Children. Egy. J. Hematol. 20(2): 103-137.

(2)-Palma-Carlos A.G.; Palma – Carlos M. L. and Costa A. C. (2005). Minor hemoglobinopathy: a risk factor for asthma, Allerg. Immunol. 3(5):177-82.

(3)-Galanello R. and Origa R. (2010).Beta thalassemia, Orphanet. J Rare Dis.21(5):11.

(4)-Sadeghi-Bojd S; Hashemi M. and Karimi M.(2008). Renal tubular function in patients with B–thalassemia major in Zahedan, Southeast Iran. Singapore Med.J. 49(5): 410-412.

(5)-Raiola G. Galati M. C.; Desanctis V.; Comuso N. M. and Pintor C. (2003).Growth and puberty in thalassemia major. J. Pediatr. Endocrinol. Met. 2: 259- 266.

(6)-Soliman A. T.; El-Banna N.; Abdel-Elfatah M.; Zalabani M. M. and Ansari B. M. (1998). Bone mineral density in prepubertal children with beta thalassemia .Correlation with growth metabolism :47(5):541-548.

(7)-Panigrahi I. and Agarwal S.(2007). Thromboembolic in beta thalassemia: Beyond the horizon . Thromb. Res., 120 (6): 783-792.

(8)-Chen A.C.; Peng C. T. and Wu S.F. (2006). Effect of deferiprone on liver iron overload and fibrosis in hepatitis C virus infected thalassemia. Hemoglobin. 30:209-214.

(9)-Ferrara M.; Matarese S. M. and Borrel B. (2004).Cardiac involvement in beta thalassemia major and beta thalassemia thalassemia intermedia. Hemoglobin 28:123-129.

(10)-Filosa A.; Dimaio S.; Vocca S. and Saviano A. (1997). Longitudinal monitoring of bone mineral density in thalassemia patients: Genetic structure and osteoporosis. Acta. paediatr.86: 342-346.

(11)-Tan J. A.; Tan K. L.; Omar K. Z. and et, al.,(2009). Interaction of Hb south Florida (Codonl ;GTG□ATG) and HbE, with beta thalassemia (IVSI-I;G>A): expression of different clinical phenotypes. EUR. J. Pediatr.,168(9):1049-54.

(12)-Cao A. and Galanello R. (2010). Beta thalassemia, Genet. Med. 12(2):61-76.

(13)-Italia K. Y.; Jijina F.Y. ; Merchant R. and *et. al.,* (2009). Response to hydroxyurea in beta thalassemia major and intermedia: experience in western .India Clin. Chim. Acta.407(1-2):10-5.

(14)-Lekawanvijit S. and Chattipakorn N. (2009). Iron overload thalassemic cardiomyopathy iron status assessment and mechanisms of mechanical and electrical disturbance due to iron toxicity, Can. J. Cardiol. 25(4):213-218.

(15)-Hazirolan T.; Eldem G.; Unal S. Akpinar B.; Gumruk F.; Alibek S. and Halilogiu M. (2010). Dual-echo TFE MRI for the assessment of myocardial iron overload in beta thalassemia major patients, Diagn. Interv. Radiol. 16:59 – 62.

(16)-Bishop, N. L.; Fudy E. P.; and Schoeff L. (2000).Clinical chemistry, Priniciples and correlations 5th. Ed. Lippinocott Williams and Wilkins, Philadelphia, p.180-220.

(17)-Thomas, L., (1998). Clinical Laboratory Diagnostics. 1st ed. Frankfurt: Books Verlagsgesellschaft; p 131-138.

(18)-Muntzel, M., Thierry, H., Bernard, L. and Tilman D., (1992). Effect of Erythropoietin on Hematocrit and Blood Pressure in Normotensive and Hypertensive rats. J. Am. Soc. Nephrol: 3:182-187.

(19)-Barnett, R. N. (1973) The principle of estimation of serum calcium. Amer. J. Clin. Path. 59: 836.

(20)-Burits, C. A. and Ashwood, E. R. (1999). Tietiz textbook of clinical Chemistry .4th .ed. W.B. ,Saunders Comp. USA ,2: 1500- 1503.
(21)-Annino, J. S and Giese, R. W., (1977) Clinical Chemistry: Principles and Procedures. 4th ed. Little, Brown and Company. Boston
(22)-Daniel W. W. (1999). Biostatistics :A foundation for analysis in the health sciences.7th.ed.John Wiley. Philadelphia, p 83.
(23)-Argyropoulou, M.I, Metafratzi, Z., Kiortsis, D.N., Bitsis, S., Tsatsoulis, A. and Efremidis, S. (2000). T_2 Relaxation Rate as an
Index of Pituitary Iron Overload in Patients with β-thalassemia Major. AJR: 175: 1567-1569.
(24)-Meral, A, Tuncel, P., Surmen, E.**,** Ozbek**,** R., Ozturk, E., and Gunay, U., (2000). Lipid Peroxidation and Antioxidant Status in ***β*** thalassemia: Pediatric Hematology and Oncology, 17:687- 693.
(25)-Mahachoklertwattana, P., Sirikulchayanonta, V., Chuansumrit, A., Karnsombat, P., Choubtum, L., Sriphrapradang, A.,
Domrongkitchaiporn, S., Sirisriro, R., and Rajatanavin, R., (2003).Bone Histomorphometry in Children and Adolescents with β- thalassemia Disease: Iron Associated Focal Osteomalacia. J. Clin.Endocrinol Metab. 88(8):3966-3972.
(26)-Ghone R. A.; Kumbar K. M. Suryakar A. N. Katkam R. V and Joshi N. G. (2008). Oxidative stress and disturbance in antioxidant balance in beta thalassemia major. Indian J. Clin. Biochemistry. 23(4):337-340.
(27)-Kassab-Chekir A.; Laradi S.; Ferchichi S.; Haj Khelil A. ; Feki M.; Amir F. and *et al.*,(2003). Oxidant, antioxidant status and metabolic data in patients with beta thalassemia . Clin. Chim. Acta.338(2):79-86.
(28)-Widad N. M. Al-Naama L. and Meaad (2003).Trace element in patients with beta thalassemia major. Haem. 6(3):376-383.
(29)-William C. and Waikan (2001). Prospects for research in hematologic disorders: Sickle cell disease and thalassemia . JAMA. 285: 640-642.
(30)-Turgeon M. L. (2005). Clinical hematology theory and procedure, 4th. Ed., lippincott Williams and Wilkins. New York. p45.
(31)- Rioja L. ;Girot R. ; Garadian M. And Cournot-witmer G.(1990). Bone disease in children with homozygous B-thalassemia , 8,Issue I: 69-86.
(32)-Rasheed N. E. and Ahmed S. A. (2009).effect of beta thalassemia on some biochemical parameters Middle East J. of family Med. (7) Issuez: 1-5.
(33)-Tantway A. A.; El Kholy M. ; Moustafa T. and elsedfy H. H. (2008). Bone mineral density and calcium metabolism in adolescent with beta thalassemia major. Pediatr. Endocrinol. Rev. 6 Suppl. 1:132-137.
(34)-Claud - Bennt J. and Pulm F. (1996). Cecil text book of medicine. 20th. W. B. Saunder. Comp. Philadelphia ,vol. 1 p.539-547.
(35)-Walter P.B.; Maklin E.A.; Porter J. and *et al.*, (2008) Inflammation and oxidant-stress in beta thalassemia patients treated with iron chelators deferasirox (ICL670) or deferoxamine: an ancillary study of the Novartis (ICL^&)AO107 trial.) Haematologica 93: 87-825.
(36)- Barton, J. C. (2007). Chelation therapy for iron overload. Curr. Gastroenterol. ReP. 9: 74-82.
(37)-Voskaridou E.; Kyrtsonis M. C.; Terpos E. and Skordili M. (2001).Bone resportion is increased in young adults with thalassemia major. Br. J. Haematol. 112:36-41.

(38)-El-Edel R. H.; Ghonaim M. M.; Abo-Salem O. M. and El-Nemr F. M.(2010). Bone mineral density and vitamine D receptor polymorphism in beta thalassemia major. Pak. J. Pharm. Sci. 23(1): 89-96.
(39)-Aleem A, Almomen, A., Al-Harakati, M.S., Hassan A., and Al- Fawaz I. (2000). Hypocalcemia Due to Hypoparathyroidism in β-thalassemia Major Patients. Ann. Saudi. Med. 20(5-6):364- 366.
(40)-Yazigi, A., Maalout, G., Khoriati, A.I., Tamim, H., and Saab, C., (2002). Bone Mineral Density in Beta-thalassemic Lebanese Children: J. Musculosked neuron interact 2(5): 463-468.