Annals of R.S.C.B., ISSN:1583-6258, Vol.28, Issue 1, 2024, Pages. 186 - 195

Received 15 February 2024; Accepted 30March 2024.

Assessment of Liver Enzymes, Calcium, and Ferritin in Beta- Thalassemia Patients

Aqdas Mohammed Sfaieh [1] *, Tamara Hmed Ahmed [2], Asmaa Musead Salih Alkinani [3]

[1] Department of Pathological Analysis, College of Science, University of Wasit, Wasit, Iraq

[2] Department of Physiology, College of Veterinary Medicine, University of Wasit, Wasit, Iraq

[3] Department of Biology, College of Science, University of Wasit, Wasit, Iraq

Emails: aaltaiy@uowasit.edu.iq [1], tahmed@uowasit.edu.iq [2], amesad@uowasit.edu.iq [3]

Abstract

This study attempts to estimate the association between liver enzyme, ferritin level and blood calcium level in patients suffering from β-thalassemia. For this purpose, a total of 50 healthy individuals without hereditary blood disorders and 75 patients with beta-thalassemia attended to at the Thalassemic Centre of in Al Kut city (Iraq) during September (2023) to the March (2024) were subjected for data collection and blood draining. In this study, we observed that a significant increase in the average levels of liver enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and serum alkaline phosphatase (ALP)], and the levels of ferreting concentration, in the patients as compared to the healthy people. In terms of AST, ALT, and ALP, in intermedia and major β-thalassemia patient were significant increases ($P<0.05$) than in healthy individuals. However, there no significant changes ($P>0.05$) were detected in minima β-thalassemia than in healthy individuals in all age groups. As for serum calcium in minima, intermedia and major β-thalassemia patient were significant decrease ($P<0.05$) in patients than in healthy individuals. This study concluded that there were elevated levels in liver enzymes indicate that β-thalassemia patients are at increased risk of heart and liver dysfunction. Also elevated ALT is affected by increasing iron overload, as reflected by elevated serum ferritin. The patients with β-thalassemia will experience hypoparathyroidism where the absence of PTH synthesiswill decreasethe gastrointestinal tract's calcium absorption and lower serum calcium levels.

Keywords: ALP, ALT, AST, Minima, Major, Iraq

Introduction

Beta-thalassemia (β-TM) was an inherited autosomal recessive illness caused by either decrease in or lack of β-globin chain production (Bajwa and Basit, 2022). It was a major health problemsthat hemoglobin levelswere below normal (Patel et al., 2018). The disease is divided into 3 types based on clinical forms are minima, results from a deficiency in a single chain, the patients in this types was asymptomatic, but have a simple anemia on normal blood tests (Akhavan-Niaki et al., 2011; Martin and Thompson, 2013), and the other type was intermediate thalassemia, which was an intermediate similarities between minor and major type, where patients may normal lives, but required occasional blood transfusion during pregnancy and illness (May et al., 2018). In β-thalassemia major types, patient required many regular blood transfusion tonormal lives due to suffer from severe bone marrow hypertrophic swelling and anemia, and the symptom do not occur at child's birth, while appears through the first two years of the child life (Martin and Thompson, 2013). Many laboratory

Received 15 February 2024; Accepted 30March 2024.

investigations such as genetic analysis, blood film, iron studies, full blood count, hemoglobin electrophoresis and prenatal testing can be used for diagnosed of β-thalassemia (AlQahtani et al., 2018). Most of the mortality and morbidity associated with β-thalassaemia caused by iron overload (Gardenghi et al., 2007). Iron overload in β-thalassaemia were responsible by two factors. Excess intestinal absorption of iron because of the erythroid hyperplasia in spite of tissue iron overload and transfusion siderosis with up to 200 mg iron was given per packed RBC unit (Rund and Rachmilewitz, 2005). Ferritin was the iron storage protein, play a key role in iron metabolism. It wascapacity to sequester the iron, the ferritin serves function as both as of iron reserve and iron detoxification (Hoffbrand et al., 2005). During the last years, liver diseaseshave emerged as a major reason of mortality in patient with β-thalassaemia major. Liver damage has been linked to β-thalassaemia insufficiently characterized; despite it was clinical relevance (Perifanis et al., 2005). Liver disease in the patients with β-thalassaemia can manifest as hepatomegaly, increased AST and ALT activities, hepatitis B and C. Fibrosis was significant frequent and it was progression is mostly influence by iron excess which may be attributable to hypertransfusion, inadequate chelation, erythrocyte catabolism and excessive iron absorption from the gut as a consequence of ineffective erythropoiesis. The major storage sites for body iron were hepatocytes, so with iron overload, this cells were relentlessly bombarded by reactive oxygen species (ROS) and eventually die. Hepatocytes are damage and start to accumulate within a years of commencing transfusion treatment after as few as 10–20 transfusion (Bonkovsky, 1991; Porter, 2001).

Many studies were reported that the prevalence of hypocalcemia (decrease of calcium in blood) in β-thalassemia major patient increase from 21-41% from 2000 to 2008 (Najafipour et al., 2008). In 2012, the incidence of hypocalcemia in patient with β-thalassemia was reported to be at 22% (Mirhosseini et al., 2013). There was a correlation between their ferritin levels with hypocalcemia in patients with β thalassemia. However ferritin level wasunrelated to vitamin D and PTH level (Adil et al., 2012). This study attempts to identify the relationship among liver enzymes, their ferritin level and blood calcium level in β-thalassemia patients.

Subjects and Methods

Samples

The present study was conducted during the period from the September (2023) to the March (2024) at the Thalassemic Centre of in AL Kut city in Iraq. A total of 75 β- thalassaemia patients; 25 patients for each group of minor, intermediate and major β- thalassaemia in addition to 50 healthy individuals as a control group. Each of the patients and control groups were divided into age group. The control individuals were closely as the same ages as the patients.

Approximately, 3 ml of the blood samples were transported into sterile test tubes, remaining for thirty minutes in incubator, then the serum separation by centrifuged. Separating a part ofthe clear serum into a sterile Eppendorf tube and kept at -20°C for measurement of serum ferritin and calcium levels. The remaining serum is used for estimation of the liver enzymes (AST, ALT and ALP).

Biochemical analysis

The ROCHE 9180 (Electrolyte Analyzer) was used to examined the amount of calcium levels by using the ion selective electrode method. Ferritin levels in the serum of all individuals

Received 15 February 2024; Accepted 30March 2024.

included in this study were determined and measured by ELISA. The measurement of serum AST and ALT was determined by colorimetric method (Randox, UK). The serum ALP was determined also by colorimetric method (Biomerieux, France), (Kind PRN and King, 1957; Bergmeyer and Bernt, 1974).

Statistical Analysis

All obtained data were collected and analyzed using of the Statistical Package for the Social Sciences (SPSS) program, specifically version 18 was used for statistical analysis. The quantitative data was reported a Mean ± Standard Deviation (M ± SD) at a significance difference value of P<0.05 (Gharban et al., 2023).

Result

The current study showed that there were significant increases (P<0.05) in the mean value of AST in patients with intermedia and major β-thalassemia than in control group in all age groups .However, there no significant changes (P>0.05) were detected in patients with minima β-thalassemia than in healthy individuals in all age groups. While there no significant changes (P>0.05) were detected between patients with intermedia and major β-thalassemia (Table 1).

Table (1): Results of AST in different clinical forms of β-thalassemia patients according to different age groups

Age group (Year)	**AST (U/l)**			
	Control	**Clinical form**		
		Minima	**Intermedia**	**Major**
5-20	24.9 ± 2.16 Aa	28.28 ± 2.81 Aa	76.14 ± 20.21 Ba	82.57 ± 12.54 Ba
21-35	22.42 ± 3.52 Aa	35.7 ± 7.05 Aa	85.85 ± 22.52 Ba	108.43 ± 25.19 Ba
36-50	22.85 ± 2.46 Aa	23.5 ± 1.77 Aa	70.85 ± 14.75 Ba	74.85 ± 19.58 Ba

Data= Mean + S. E. M.

Different capital letters refer to significant differences between groups (P≤0.05).

Different small letters refer to significant differences within groups (P≤0.05).

Similar capital and small letters refer to non-significant differences (P>0.05)

The results showed that there were significant increases (P<0.05) in the mean value of ALT in patients with intermedia and major β-thalassemia than in control group in all age groups. However, there no significant changes (P>0.05) were detected in patients with minima β-thalassemia than in healthy individuals in all age groups. While there no significant changes (P>0.05) were detected between patients with intermedia and major β-thalassemia (Table 2).

Table (2): Results of ALT in different clinical forms of β-thalassemia patients according to different age groups

Age group (Year)	**ALT (U/l)**	
	Control	**Clinical form**

Annals of R.S.C.B., ISSN:1583-6258, Vol.28, Issue 1, 2024, Pages. 186 - 195

Received 15 February 2024; Accepted 30March 2024.

		Minima	**Intermedia**	**Major**
5-20	22.85 ± 2.26 Aa	24.5 ± 1.89 Aa	75.85 ± 10.95 Ba	85.85 ± 23.39 Ba
21-35	23.7 ± 2.98 Aa	24 ± 3.81 Aa	87.42 ± 29.61 Ba	100.14 ± 25.74 Ba
36-50	21.7 ± 3.05 Aa	23 ± 2.85 Aa	88 ± 22.8 Ba	94.28 ± 26.53 Ba

The results revealed that there were significant increases (P<0.05) in the mean value of ALP in patients with intermedia and major β-thalassemia than in control group in all age groups. However, there no significant changes (P>0.05) were detected in patients with minima β-thalassemia than in healthy individuals in all age groups. While there no significant changes (P>0.05) were detected between patients with intermedia and major β-thalassemia (Table 3).

Table (3): Results of ALP in different clinical forms of thalassemia patients according to different age groups

Age group (Year)	**ALP (U/l)**			
	Control	**Clinical form**		
		Minima	**Intermedia**	**Major**
5-20	129.86 ± 18.56 Aa	139.2 ± 16.76 Aa	364.43 ± 42.99 Ba	386.14 ± 52.72 Ba
21-35	133.43 ± 18.82 Aa	135.8 ± 21.68 Aa	333.43 ± 64.67 Ba	465.29 ± 48.78 Ba
36-50	116.4 ± 13.32 Aa	156.86 ± 23.89 Aa	436.71 ± 44.41 Ba	453 ± 47.33 Ba

The results recorded that there were significant increases (P<0.05) in the mean value of calcium in patients with minima, intermedia and major β-thalassemia than in control group in all age groups .However, there no significant changes (P>0.05) were detected between patients with minima, intermedia and major β-thalassemia (Table 4).

Table (4): Results of calcium in different clinical forms of thalassemia patients according to different age groups

Age group (Year)	**Ca(mg/ dl)**			
	Control	**Clinical form**		
		Minima	**Intermedia**	**Major**
5-20	10.48 ± 0.46 Aa	4.05 ± 0.51 Ba	4.11 ± 0.4 Ba	4.82 ± 0.91 Ba
21-35	11.17 ± 0.43 Aa	4.68 ± 0.86 Ba	3.93 ± 0.95 Ba	4.49 ± 1.1 Ba
36-50	10.26 ± 0.38 Aa	4.85 ± 0.75 Ba	5.42 ± 0.53 Ba	4.01 ± 0.76 Ba

Received 15 February 2024; Accepted 30March 2024.

The results reported that there were significant increases (P<0.05) in the mean value of ferritin in patients with minima, intermedia and major β-thalassemia than in control group in age group (5-20). Also there were significant increases (P<0.05) between intermedia and major β-thalassemia in age group (21-35) years. However, there significant increases (P<0.05) were detected between patients with minima, intermedia, major β-thalassemia and control groups in age group (36-50) years (Table 5).

Table (5): Results of ferritin in different clinical forms of thalassemia patients according to different age groups

Age group (Year)	Ferritin (ng/ml)			
	Control	Clinical form		
		Minima	Intermedia	Major
5-20	115.2 ± 11.8 Aa	1117.7 ± 121.1 Ba	1207.7 ± 164.6 Ba	1473 ± 233.9 Ba
21-35	129.7±11.83 Aa	1814.3±153.2 Ba	2111.7 ± 201.3 Bb	3107.0 ± 310.2 Cb
36-50	115.8±12.18 Aa	2048.3±273.6 Bb	3729.3 ± 294.2 Cc	4987.3 ± 370.5 Dc

Discussion

In this study, we observed that levels of liver enzymes (AST, ALT, and ALP) were increased significantly in β-thalassemia patients as detected by various studies nationally (Jwaid and Gata, 2020; Abd and Al Samarrai, 2022; AL-hameedawi and Al-Shawi, 2022) and internationally (De et al., 2019; Heris et al., 2021; Saravani et al., 2024). However, difference in the obtained results may be due to the difference in sampling, inclusion and exclusion criteria, control of confounders and sample size. Sobhani et al. (2019) determined that the range of liver enzyme and serum ferritin level, which was the best time to start treatment with iron chelates in moderate β-thalassemia patients. Huang et al. (2023) reported that high ferritin having a significant relationship with increased levels of liver enzymes, which are completely consistent with the findings of our study and show a positive correlation to ferritin and liver enzymes. Un-transported iron (free iron) has been evaluated as an indicator of iron overload in β-thalassemia patients (Saravani et al., 2024).

It has been showed that the level of serum ferritin compared to free iron in the blood gives a better prognosis than the accumulated iron in the liver (Anastasiou et al., 2017). A study was conducted on 50 β-thalassemia patients and 50 age- and sex-matched control groups to investigate the change of serum liver enzymes and also whether this change can be related to common hematological indicators (De et al., 2019). Iron overload in β-thalassemia patients may occur as a result of frequent transfusion therapy, ineffective erythropoiesis, and compensatory absorption of iron from the small intestine due to anemia (Taher and Saliba, 2017). Studies in patients with transfusion-independent β-thalassemia and thalassemia mice with moderate anemia have shown increased iron absorption from the intestine (Lertsuwan et al., 2018; Di Modica et al., 2022). Therefore, upregulation of ferroportin and downregulation of hepcidin may increase intestinal iron absorption and lead to iron overload in β-thalassemia (Camaschella et al., 2020). Iron chelators are commonly used to reduce iron overload in β-

Received 15 February 2024; Accepted 30March 2024.

thalassemia patients (Mobarra et al., 2016). However, urine calcium and serum calcium decreased immediately after iron chelators treatment; therefore, it was hypothesized that the reduction in serum calcium levels due to the reduction in aluminum levels during iron chelators treatment may indirectly be due to increased calcium deposition in bone (Kontoghiorghes et al., 2020; Tanous et al., 2021).

Studies of prepubertal children (8 to 9 years) have shown that β-thalassemia patients have significantly reduced bone mineral density (BMD) compared to age- and sex-matched controls (El-Nashar et al., 2017; Hamidi et al., 2019). Similarly, a study of children and adolescents (8 to 25 years) with transfusional and non-transfusional β-thalassemia found that bone formation and growth, thickness, bone pain, and shortness of breath were reduced in most patients (Porter et al., 2021). The incidence of fractures in patients with all types of β-thalassemia is approximately 12% and is equally distributed between males and females (Dede et al., 2016). One possible cause of decreased bone density due to osteopenia/osteoporosis may be an imbalance in the bone remodeling process (Jeney, 2017). This had a strong correlation with reduction in BMD and patient survival. Also, osteoclastic factors can also inhibit osteoblast differentiation and activity, thereby reducing bone formation (Kim et al., 2020). In addition to imbalance in bone remodeling, hypocalcemia is also reported in animals and patients with thalassemia, possibly due to decreased calcium absorption (Wong et al., 2016; Yang et al., 2020). Significant reductions in intestinal calcium absorption have been seen in critically ill β-thalassemia patients with low bone density (De Sanctis et al., 2018).

Beggs and Alexander (2017) showed that calcium absorption by the epithelium of the small intestine is significantly reduced. This phenomenon is present in both sexes, and daily injections of vitamin D3 at 1 μg/kg effectively increase absorption of calcium, while maintaining normal intestinal tract in β-thalassemia patients. One study had shown that intracellular calcium transporters and calcium transport-related proteins are down-regulated. This damage can reduce calcium absorption in the intestines (Phoaubon et al., 2021). Lertsuwan et al. (2018) of pernicious anemia have shown a reduction in jejunal NKA activity that is essential for stabilization of intracellular Na+ that necessary for calcium absorption. Decreased albumin levels and iron deficiency originate from different organ systems, suggesting that calcium may be reduced with anemia (Boronat et al., 2017). Hormones play a role in regulating calcium absorption in the intestine; therefore, deficiency or abnormalities of these hormones can significantly affect calcium transport in the intestine in many diseases as thalassemia (Diaz and Suárez-Ortega, 2018). Expression of D3 receptors in duodenal epithelial cells is reduced in β-thalassemia. In addition to β-thalassemia, patients with sickle cell disease also have low levels of D3 in their serum (Charoenphandhu et al., 2019). Decreased serum D3 levels in β-thalassemia patients and animals lead to reduced serum calcium levels and BMD, and impaired production and function of D3 contributes to absorption of calcium in β-thalassemia (Bajoria et al., 2019; Rouf Moustafa et al., 2023). PTH increases serum calcium concentration by increasing absorption of calcium from intestine; however, low levels of PTH, which is known to stimulate bone resorption, lead to a decrease in BMD (Rejnmark and Ejlsmark-Svensson, 2020).

Conclusions

Received 15 February 2024; Accepted 30March 2024.

Elevated levels of liver enzymes indicate that β-thalassemia patients are at increased risk of heart and liver dysfunction. It seems that patients with β-thalassemia suffer from liver damage in the long term. In our study, due to defects in the medical records, it was not possible to check the total protein, which requires a more detailed investigation in future studies. The results recorded that serum ferritin level was a significant correlated with the liver enzymes.

References

[1] Abd, H. M., and Al Samarrai, O. R. (2022). Evaluation of hepcidin, ferritin and iron levels with liver enzymes of β-thalassemia patients in Diyala governorate, Iraq. In *AIP Conference Proceedings* (Vol. 2450, No. 1). AIP Publishing.

[2] Adil A, Sobani ZA, Jabbar A, Adil SN, Awan S(2012). Endocrine complications in patients of beta thalassemia major in a tertiary care hospital in Pakistan. *J Pak Med Assoc*. 2012; 62:307–10.

[3] Akhavan-Niaki H, Derakhshandeh-Peykar P, Banihashemi A, Mostafazadeh A, Asghari B, Ahmadifard MR, Azizi M, Youssefi A, Elmi MM. A comprehensive molecular characterization of beta thalassemia in a highly heterogeneous population. Blood Cells Mol Dis.(2011) 15;47(1):29-32.

[4] AL-hameedawi, A. K. J., and Al-Shawi, A. A. (2022). Clinical Evaluation of Some Biochemical and Hematological Variables and Their Relationship to Serum Ferritin Levels of Major Beta Thalassemia Patients in Maysan Governorate-Iraq. *Journal of Kufa for Chemical Sciences*, *2*(9), 436-462.

[5] AlQahtani RS, Bedaiwi AA, Alburkani AM, AlFahed MM, Alhoraibi RA, Tarawah AM: Knowledge and response of the community to premarital screening program (Sickle Cell Anemia\Thalassemia); AlMadinah, Saudi Arabia. J ApplHematol. 2018, 9:59. 10.4103/joah.joah_1_18

[6] Anastasiou, O. E., Kälsch, J., Hakmouni, M., Kucukoglu, O., Heider, D., Korth, J., and Canbay, A. (2017). Low transferrin and high ferritin concentrations are associated with worse outcome in acute liver failure. *Liver International*, *37*(7), 1032-1041.

[7] And Liver Enzymes With Serum Ferritin In Beta Thalassaemia Major". (2018) vol . 17, PP 62-67

[8] Bajoria, R., Rekhi, E., Almusawy, M., and Chatterjee, R. (2019). Hepatic hemosiderosis contributes to abnormal vitamin D-PTH axis in thalassemia major. *Journal of Pediatric Hematology/Oncology*, *41*(2), e83-e89.

[9] Bajwa H, Basit H: Thalassemia. StatPearls Publishing, Treasure Island (FL); 2022.

[10] Beggs, M. R., and Alexander, R. T. (2017). Intestinal absorption and renal reabsorption of calcium throughout postnatal development. *Experimental Biology and Medicine*, *242*(8), 840-849.

[11] Bergmeyer, H. U., and Bernt, E. (1974). Colorimetric assay of Reitman and Frankel. In *Methods of enzymatic analysis* (pp. 735-739). Academic press.

[12] Bonkovsky, H. L. (1991). Iron and the liver. *The American journal of the medical sciences*, *301*(1), 32-43.

[13] Boronat, M., Santana, A., Bosch, E., Lorenzo, D., Riaño, M., and Garcia-Canton, C. (2017). Relationship between anemia and serum concentrations of calcium and

phosphorus in advanced non-dialysis-dependent chronic kidney disease. *Nephron*, *135*(2), 97-104.

[14] Camaschella, C., Nai, A., and Silvestri, L. (2020). Iron metabolism and iron disorders revisited in the hepcidin era. *Haematologica*, *105*(2), 260.

[15] Charoenphandhu, N., Aeimlapa, R., Sooksawanwit, S., Thongbunchoo, J., Teerapornpuntakit, J., Svasti, S., and Wongdee, K. (2019). Responses of primary osteoblasts and osteoclasts from hemizygous β-globin knockout thalassemicmice with elevated plasma glucose to 1, 25-dihydroxyvitamin D3. *Scientific Reports*, *9*(1), 13963.

[16] De Sanctis, V., Soliman, A. T., Elsefdy, H., Soliman, N., Bedair, E., Fiscina, B., and Kattamis, C. (2018). Bone disease in β thalassemia patients: past, present and future perspectives. *Metabolism*, *80*, 66-79.

[17] De, A., Samaddar, D., Sen, A., and Sen, P. K. (2019). Comparative assessment of serum liver enzymes (AST and ALT) in thalassemia patients of Murshidabad and matched controls. *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, *17*(11), 01-05.

[18] Dede, A. D., Trovas, G., Chronopoulos, E., Triantafyllopoulos, I. K., Dontas, I., Papaioannou, N., and Tournis, S. (2016). Thalassemia-associated osteoporosis: a systematic review on treatment and brief overview of the disease. *Osteoporosis International*, *27*, 3409-3425.

[19] Di Modica, S. M., Tanzi, E., Olivari, V., Lidonnici, M. R., Pettinato, M., Pagani, A., and Nai, A. (2022). Transferrin receptor 2 (Tfr2) genetic deletion makes transfusion-independent a murine model of transfusion-dependent β-thalassemia. *American Journal of Hematology*, *97*(10), 1324-1336.

[20] Diaz, R., and Suárez-Ortega, L. (2018). Abnormalities in Calcium Homeostasis. *Pediatric Endocrinology: A Practical Clinical Guide*, 479-496.

[21] El-Nashar, M., Mortagy, A. K., El-Beblawy, N. M., El-Gohary, E., Kamel, I. M., Rashad, M., and Mouharam, W. A. (2017). Parathyroid hormone in pediatric patients with β-thalassemia major and its relation to bone mineral density; a case control study. *Egyptian Journal of Medical Human Genetics*, *18*(1), 75-78.

[22] Gardenghi S, Marongiu MF, Ramos P, et al. Ineffective erythropoiesis in β- thalassemia is characterized by increased iron absorption mediated by downregulation of hepcidin and up-regulation of ferroportin, Blood 2007;109(11): 5035.

[23] Gharban, H. A., Al-Ghuraibawi, H. N., Al-Rubaye, Z. A., Jahlol, H. A., Al-Zergany, A. A., and Al-Abedi, G. J. (2023). Most Clinically Detected Viral Diseases in Field Animals of Wasit Province, Iraq. *Annals of the Romanian Society for Cell Biology*, *27*(01), 154-168.

[24] Hamidi, Z., Mohajeri-Tehrani, M., Hamidieh, A. A., Naghghash, A., Behfar, M., and Alimoghaddam, K. (2019). How thalassemia affects endocrinological, BMD and bone metabolism parameters-a cross sectional study. *Ann Clin Lab Res*, *7*(1), 293.

[25] Heris, H. K., Nejati, B., Rezazadeh, K., Sate, H., Dolatkhah, R., Ghoreishi, Z., and Esfahani, A. (2021). Evaluation of iron overload by cardiac and liver T2* in β-thalassemia: Correlation with serum ferritin, heart function and liver enzymes. *Journal of cardiovascular and thoracic research*, *13*(1), 54.

[26] Hoffbrand AV, Catovsky D, Tudenham EG. Postgraduate haematology, 5th ed., Blackwell publishing 2005; 88-90.

Received 15 February 2024; Accepted 30March 2024.

[27] Huang, J. H., Li, R. H., and Tsai, L. C. (2023). Dual nature of ferritin for hematologic, liver functional, and metabolic parameters in older diabetic patients. *Scientific Reports*, *13*(1), 20207.

[28] Jeney, V. (2017). Clinical impact and cellular mechanisms of iron overload-associated bone loss. *Frontiers in pharmacology*, *8*, 242826.

[29] Jwaid, S. H., and Gata, A. M. (2020). Comparison study of major thalassemia, thalassemia intermedia of Iraqi patients and control groups for effectiveness of liver enzymes. *Medico-legal update*, *20*(1), 1181-1184.

[30] Kim, J. M., Lin, C., Stavre, Z., Greenblatt, M. B., and Shim, J. H. (2020). Osteoblast-osteoclast communication and bone homeostasis. *Cells*, *9*(9), 2073.

[31] Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Path. 1954; 7: 322-6.

[32] Kontoghiorghes, G. J., and Kontoghiorghe, C. N. (2020). Iron and chelation in biochemistry and medicine: new approaches to controlling iron metabolism and treating related diseases. *Cells*, *9*(6), 1456.

[33] Lertsuwan, K., Wongdee, K., Teerapornpuntakit, J., and Charoenphandhu, N. (2018). Intestinal calcium transport and its regulation in thalassemia: interaction between calcium and iron metabolism. *The journal of physiological sciences*, *68*, 221-232.

[34] Martin, A., and Thompson, A.A., "Thalassemias*Pediatric Clinics"*.(2013) *60* 1383–1391.

[35] May C Chien, MD; Chief Editor: Vikramjit S Kanwa ", Thalassemia Intermedia". Dec 24, 2018.

[36] Mirhosseini NZ, Shahar S, Ghayour-Mobarhan M, Banihashem A, Kamaruddin NA, Hatef MR, Esmaili HA (2013). Bonerelated complications of transfusiondependent beta thalassemia among children and adolescents. *J Bone Miner Metab*. 31(4): 468–76

[37] Mobarra, N., Shanaki, M., Ehteram, H., Nasiri, H., Sahmani, M., Saeidi, M., and Azad, M. (2016). A review on iron chelators in treatment of iron overload syndromes. *International journal of hematology-oncology and stem cell research*, *10*(4), 239.

[38] Najafipour F, Aliasgarzadeh A, Aghamohamadzadeh N, Bahrami A, Mobasri M, Niafar M, Khoshbaten M (2008). Across-sectional ttudy of metabolic andSaudi Medicine. 20(5): 364–6

[39] Patel, S. A., Siddiqui, A. M., and Kareem, I. (2018). Correlative study of serum bilirubin and liver enzymes with serum ferritin in beta thalassaemia major. *IOSR-JDMS*, *1*, 62-67.

[40] Perifanis V, Tziomalos K, Tsatra I, Karyda S, Patsiaoura K, Athanassiou-Metaxa M. Prevalence and severity of liver disease in patients with beta thalassemia major. A single-institution fifteen year experience. Haematologica. 2005; 90: 1136-8.**.**

[41] Phoaubon, S., Lertsuwan, K., Teerapornpuntakit, J., and Charoenphandhu, N. (2021). Hepcidin induces intestinal calcium uptake while suppressing iron uptake in Caco-2 cells. *Plos one*, *16*(10), e0258433.

[42] Porter JB. Practical management of iron overload. Br J Haematol. 2001; 115(2): 239-52.

[43] Porter, J., Taher, A., Viprakasit, V., Kattamis, A., Coates, T. D., Garbowski, M., and Cappellini, M. D. (2021). Oral ferroportin inhibitor vamifeport for improving iron homeostasis and erythropoiesis in β-thalassemia: current evidence and future clinical development. *Expert Review of Hematology*, *14*(7), 633-644.

Received 15 February 2024; Accepted 30March 2024.

[44] Rejnmark, L., and Ejlsmark-Svensson, H. (2020). Effects of PTH and PTH hypersecretion on bone: a clinical perspective. *Current osteoporosis reports*, *18*, 103-114.

[45] Rouf Moustafa, S., Kadhem Al-Hakeim, H., Alhillawi, Z. H., and Maes, M. (2023). In Transfusion-Dependent Thalassemia Children, Increased Iron Overload is Associated with Lower Serum Alpha-Klotho, Which is Strongly Associated with Lower Total and Ionized Calcium Concentrations. *Current Molecular Medicine*, *23*(5), 442-452.

[46] Rund D, Rachmilewitz E. β-thalassemia, N Eng J Med. 2005; 353: 1135-1139.

[47] Saravani, K., Ostadrahimi, P., and Jahanifard, A. (2024). Evaluation of the level of liver enzymes and its relationship with ferritin and the frequency of blood transfusion in patients with thalassemia. *Cellular, Molecular and Biomedical Reports*, *4*(2), 100-110.

[48] Sobhani, S., Rahmani, F., Rahmani, M., Askari, M., and Kompani, F. (2019). Serum ferritin levels and irregular use of iron chelators predict liver iron load in patients with major beta thalassemia: a cross-sectional study. *Croatian medical journal*, *60*(5), 405-413.

[49] Taher, A. T., and Saliba, A. N. (2017). Iron overload in thalassemia: different organs at different rates. *Hematology 2014, the American Society of Hematology Education Program Book*, *2017*(1), 265-271.

[50] Tanous, O., Azulay, Y., Halevy, R., Dujovny, T., Swartz, N., Colodner, R., and Levin, C. (2021). Renal function in β-thalassemia major patients treated with two different iron-chelation regimes. *BMC nephrology*, *22*, 1-11.

[51] Wong, P., Fuller, P. J., Gillespie, M. T., and Milat, F. (2016). Bone disease in thalassemia: a molecular and clinical overview. *Endocrine reviews*, *37*(4), 320-346.

[52] Yang, W. P., Chang, H. H., Li, H. Y., Lai, Y. C., Huang, T. Y., Tsai, K. S., and Shih, S. R. (2020). Iron overload associated endocrine dysfunction leading to lower bone mineral density in thalassemia major. *The Journal of Clinical Endocrinology and Metabolism*, *105*(4), e1015-e1024.