

## Fibroblasts Growth Factor 23 (FGF23) Level and its Correlation with Serum Calcium, Phosphorus, and Ferritin Level in Adult $\beta$ -Thalassemia Patients: Single Center Study

RASHA RAWI, M.D.\*; MOHAMED A. MORAD, M.D.\*; DOAA MOSTAFA, M.D.\*\*\*;  
MAHA F. YACOUB, M.D.\* and HEND A. ELSHEIMY, M.D.\*\*

*Hematology Unit, Internal Medicine Department\*, Internal Medicine Department\*\* and Department of Clinical & Chemical Pathology\*\*\*, Faculty of Medicine, Cairo University*

### Abstract

**Background:** Thalassemia Patients frequently experience complications like bone affection and damage. Serum fibroblast growth factor (FGF23)/thalassemia bone disease development is influenced by FGF23. Correlation of FGF23 with serum Calcium (Ca), phosphorus (Ph) and Ferritin level in thalassemia patients is an important step to investigate and treat thalassemia bone disease.

**Aim of Study:** To assess the FGF-23 level in the serum of adult  $\beta$ -thalassemia patients and its correlation to ferritin, calcium, and phosphorus levels.

**Patients and Methods:** Fifty-three patients with  $\beta$ -thalassemia; transfusion dependent thalassemia (TDT) and non-transfusion dependent (NTDT) were included. Any patient with chronic inflammation, renal impairment, or failure were excluded. Serum ferritin, phosphorous, calcium, and FGF-23 were checked and analyzed.

**Results:** Mean age of the studied population was  $30 \pm 7.6$  years, 37.3% males and 62.3% females. TDT were 41.5% & NTDT were 58.5%. Patients suffering from Bony pains were 54.7% and cardiac complications were 18.9%.

Significant correlations were found between serum FGF-23 and each of Bony pains and Cardiac complications ( $p$ -value  $< 0.001$ ).

Significantly negative correlations between serum FGF-23 and each of serum Ferritin & Ca ( $p$ -value  $< 0.001$ ). Additionally, FGF-23 and serum Ph level have a strong positive correlation ( $p$ -value  $< 0.001$ ).

**Conclusion:** FGF-23 was significantly correlated with (Ferritin, Ca, & Ph levels), Suggesting its participation in Ca homeostasis and thalassemia bone disease. Further studies with larger number of patients is required for more understanding to FGF-23 role in thalassemia and its complications.

**Key Words:**  $\beta$ -thalassemia – FGF-23 – Ferritin – Bone disease.

### Introduction

**REPEATED** blood transfusions and iron excess are features of the inherited hemolytic anemia known as thalassemia. Concomitant iron chelation is necessary to prevent the problems of iron excess since the body has a limited capacity to eliminate iron [1]. Occurrence of complications in thalassemia patients could be due to repeated blood transfusions, iron overload, or chelation therapy.

The combined effects of chronic anemia and iron overload can lead to multiple end-organ complications such as cardiomyopathy, increased risks of blood-borne, liver, pituitary, and bone diseases [2]. In fact, thalassemia bone disease is one of the most important and common associated complications.

There is still much to learn about thalassemia bone disease. An interplay of risk factors that include genetic factors, hormonal deficiency, bone marrow expansion, iron toxicity, chelators, skeletal dysmorphism and increased turnover of bone, may be implicated [1].

The high prevalence of bone disease in transfusion-dependent thalassemia is seen in both younger and older patients as life expectancy continues to improve [1].

There are 22 members of the fibroblast growth factor (FGF) family, each with a distinct function [3]. In bone cells, a protein known as fibroblast growth factor 23 is created using instructions from the FGF-23 gene. This protein is necessary in phosphate homeostasis. Phosphorus has a variety of purposes, but among them, it is essential for the development and growth of bones in children as well as for maintaining bone strength in adults [4].

**Correspondence to:** Dr. Rasha Rawi,  
[E-Mail: rasharawi@kasralainy.edu.eg](mailto:Rasharawi@kasralainy.edu.eg)

FGF23 is functionally unique from other members of this family because it suppresses 1-hydroxylase activity in the kidney and operates primarily as a phosphaturic factor [5]. The kidneys are instructed by fibroblast growth factor 23 to halt reabsorbing phosphate into the circulation [6].

Recent investigations have discovered connections between FGF23 and other iron status markers. Among them is the inverse relationship between FGF23 and serum iron [7], hemoglobin (Hb) [8], and ferritin [9].

In this study we aim to assess FGF23 in the serum of adult  $\beta$ -thalassemia patients and detect its correlation to serum ferritin, calcium & phosphorus levels.

### Patients and Methods

In this case-control study, 53 adult  $\beta$ -thalassemia patients (TDT & NTDT) diagnosed by hemoglobin electrophoresis were enrolled. Patients were recruited from Kasr Al-Ainy Clinical Hematology Department and Clinic from May 2020-May 2022.

Written informed consent was taken from all subjects. The study was approved by the ethical committee. Any adult patient with beta thalassemia and aged more than 14 years was included. Patients with chronic inflammatory condition, renal impairment or renal failure were excluded from the study.

All subjects were subjected to full history taking and complete physical examination. Laboratory investigations were done in the form of complete blood count (CBC), liver function test, renal function tests, Hepatitis C virus Antibody (HCV-Ab), Serum Ferritin, serum phosphorous, calcium.

FGF23 was measured in serum of all patients using Ray-Biotech\* ELISA kit. An anti-human FGF-23 antibody is used in the FGF-23 assay, which is coated on a 96-well plate. Standards and samples are pipetted into the wells and FGF-23 present in a sample is bound to the wells by the immobilized antibody. The wells are washed, and biotinylated anti-human FGF-23 antibody is added. Streptavidin that has been HRP-conjugated is pipetted into the wells after unbound biotinylated antibody has been washed away. The wells are cleaned once again, a TMB substrate solution is added, and color develops in accordance to the amount of FGF-23 bound to the substrate. At 450 nm, the color intensity is determined after the stop solution converts blue to yellow [10].

### Statistical methods:

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 26 (IBM Corp., Armonk, NY, USA). Data were summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Mann-Whitney test. For comparing categorical data, Chi-square ( $\chi^2$ ) test was performed. Exact test was used instead when the expected frequency is less than 5. Statistics were considered significant for  $p$ -values less than (0.05).

### Results

The study included 53 adult  $\beta$ -thalassemia patients, twenty males (37.3%) and 33 females (62.3%). The mean age of the studied population was  $30 \pm 7.6$  years. Twenty-two (41.5%) were TDT patients while 31 (58.5%) were NTDT.

Thirty one patients were receiving chelation (58.5%), while 22 patients were not receiving chelation (41.5%).

Bony pains were presented in 29 (54.7%); 10 (34.5%) patients were TDT while 19 (65.5%) patients were NTDT and bony pains was absent in 24 (45.3%). Splenectomy was done in 21 (39.6%) patients, 16 of them were TDT (76.2%) and remaining 5 patients (23.8%) were NTDT. Cardiac complications were present in 10 patients (18.9%) and diabetes mellitus in 11 (20.8%). Anti HCV-Ab was positive in 13 (24.5%) patients (treated patients).

Table (1): Descriptive analysis of the variables.

Value	Mean $\pm$ Std. Deviation	Minimum	Maximum
Age in Years	30.1 $\pm$ 7.57	14	55
Hemoglobin	9.06 $\pm$ 1.7	5.70	13.70
Platelets	449.17 $\pm$ 239.58	133	926
Total Leucocytic Count	9.32 $\pm$ 3.30	5.10	22.20
Creatinine	0.67 $\pm$ 0.25	0.20	1.90
Urea	27.53 $\pm$ 15.39	14	119
Alanine Transaminase	28.76 $\pm$ 9.77	7	52
Ferritin	1029.26 $\pm$ 1304.25	30	8935
Serum Calcium	9.06 $\pm$ 0.596	6.70	9.90
Serum Phosphorus	4.03 $\pm$ 0.72	2.1	5.3
FGF-23 in U/ml	168.62 $\pm$ 42.92	95	312

- Reference values of lab results are; Total Leucocytic Count ( $4-11 \times 10^3$ ), Hemoglobin (12-15g/dL), Platelets ( $150-450 \times 10^3$ ), Urea (17-43mg/dL), Creatinine (0.6-1mg/dL), ALT (up to 35U/L), Ferritin (30-400ng/ml), Serum Ca (8.6-10.2mg/dl), Serum Ph (2.5-4.5mg/dl), FGF (29-72U/ml).

There was a significant correlation between serum FGF-23 on one hand and chelation status, Bony pains, and Cardiac complications on other hand, ( $p$ -value  $<0.001$ ).

Regarding the relation of serum FGF-23, serum Ca, serum Ph with type of thalassemia there were all of no statistical significance.

Additionally, there was a substantial inverse relationship between Ph level and bony pains. ( $p$ -value  $<0.001$ ), in addition to a significant positive correlation between bony pains and Ca level ( $p$ -value  $<0.001$ ).

Table (2): Value of FGF-23 expressed in (mean  $\pm$  Standard deviation) in each type of Thalassemia expressed.

(Mean $\pm$ SD)	Thalassemia Type	
	TDT=22 cases	NTDT=31 cases
FGF-23 in U/ml	159.36 $\pm$ 33.461	175.19 $\pm$ 47.970
Serum Ca	9.0909 $\pm$ 0.53266	9.0329 $\pm$ 0.64412
Serum Ph	3.895 $\pm$ 0.7662	4.126 $\pm$ 0.6894

TDT : Transfusion dependent Thalassemia.  
NTDT : Non transfusion dependent thalassemia.  
FGF-23: Fibroblast growth factor-23.

Table (3): Correlation of the possible factors associated with serum FGF23 in Thalassemia patients expressed.

Variable	Pearson correlation	$p$ -value
Ferritin	-0.159	$<0.001$
Serum Ca	-0.103	$<0.001$
Serum Ph	0.004	$<0.001$

## Discussion

Fibroblast growth factor 23 is a glycoprotein that is secreted primarily from the mature osteoblasts and osteocytes. FGF-23 has many actions; it is involved in iron, Calcium, and phosphate homeostasis [8,9].

Bone disease is a severe complication that affect thalassemia patients particularly transfusion dependent thalassemia. Understanding the pathogenesis thalassemia bone disease is a great step towards right assessment and better management [11,12].

This study included 53 adult  $\beta$ -thalassemia patients; 37.3% males and 62.3% females with mean age was 30 $\pm$ 7.6 years in the studied populations. TDT patients represented 41.5% of the study population, while 58.5% were NTDT.

Our results stated that 54.7% of patients involved were complaining of bony pains and this

agrees with previous studies, where bony pain was present in 34% of adult  $\beta$  thalassemia patients [13].

Eighteen point nine percent of our patients were complaining of Cardiac complications, a significant positive correlation that is found between FGF-23 level and occurrence of cardiac complications in our patients, this was In agreement with Batra et al., 2016, that reported the occurrence of many cardiac complications as ventricular hypertrophy, systemic hypertension, up to occurrence of cardiac events or mortality in association with elevated FGF-23 level [14].

According to our results we found that all our patients showed elevated FGF-23 level (168.62  $\pm$  42.92U/ml), numerous previously published studies detected an increase of serum FGF-23 level following intravenous iron infusion so it is expected to found high level of FGF-23 in thalassemia [15,16,17].

Interestingly, 9 of our patients have normal ferritin, and only 17 patients have elevated ferritin with values exceedingly more than (1000ng/ml) and despite this great variability in ferritin level among our patients, all patients experienced elevated serum FGF-23 level. Evidently this raises the possibility of involvement of other factors that control the secretion of FGF-23 in thalassemia patients.

Other factors that could be implemented in the secretion of FGF-23 may be related to morbidities that the thalassemia patients suffer from as hypocalcemia, hyperphosphatemia [11,18].

According to our results FGF-23 was significantly inversely correlated with ferritin level and significantly positively correlated with phosphorus level all agreement with Honda et al., who found the same correlations in hemodialysis patients [19]. Both Stefanopoulos et al., & Yang WP et al., also supported our results and related elevation of phosphate level in thalassemia patient due to chronic hemolysis, repeated transfusions, or occurrence of hypoparathyroidism in thalassemia patients [20,21].

However, that correlation disagreed with Yoshiko et al., that observed the decrease of phosphate level induced by increased FGF-23 due to decreased phosphate reabsorption in renal tubules [22].

Another significant inverse correlation between FGF-23 and serum calcium level was found in our results and this disagreed with Rayego-Mateos et

al that stated that there is an elevation of serum calcium associated with elevated FGF-23 level and this is regulated via other factors as parathormone, and vitamin D [23].

#### Conclusion:

Thalassemia bone disease is a major problem facing thalassemia patients. Its pathogenesis includes interplay between variable of factors.

One of the continuously appearing involved factors is FGF-23 that plays an important role in homeostasis of phosphorus and calcium. FGF-23 is variably by iron status especially in thalassemia patients.

Further studies with more patients may help us more and more to understand the correlations between FGF-23 with Ca, phosphorus and in specific the correlation between FGF-23 and iron overload.

#### References

- 1- WONG P., FULLER P.J., GILLESPIE M.T. and MILAT F.: Bone Disease in Thalassemia: A Molecular and Clinical Overview. *Endocr Rev.* Aug., 37 (4): 320-46, 2016.
- 2- PINTO V.M. and FORNI G.L.: Management of Iron Overload in Beta-Thalassemia Patients: Clinical Practice Update Based on Case Series. *Int. J. Mol. Sci.*, Nov. 20; 21 (22): 8771, 2020.
- 3- ITOH N. and ORNITZ D.M.: Evolution of the Fgf and Fgfr gene families. *Trends Genet*, 20: 563-569, 2004.
- 4- IMEL E.A. and ECONS M.J.: Fibroblast growth factor 23: Roles in health and disease. *J. Am. Soc. Nephrol.*, Sep. 16 (9): 2565-75, 2005.
- 5- SHIMADA T., MIZUTANI S., MUTO T., YONEYA T., HINO R., TAKEDA S., TAKEUCHI Y., FUJITA T., FUKUMOTO S. and YAMASHITA T.: Cloning and characterization of FGF23 as a causative factor of tumor-induced osteo-malacia. *Proc. Natl. Acad. Sci. USA*, 98: 6500-6505, 2001.
- 6- BERGWITZ C. and JÜPPNER H.: Regulation of phosphate homeostasis by PTH, vitamin D, and FGF23. *Annu. Rev. Med.*, 61: 91-104, 2010.
- 7- IMEL E.A., PEACOCK M., GRAY A.K., PADGETT L.R., HUI SL. and ECONS M.J.: Iron modifies plasma FGF23 differently in autosomal dominant hypo-phosphatemic rickets and healthy humans. *J. Clin. Endocrinol. Metab.*, Nov. 96 (11): 3541-9, 2011.
- 8- BRAITHWAITE V., JARJOU L.M., GOLDBERG G.R. and PRENTICE A.: Iron status and fibroblast growth factor-23 in Gambian children. *Bone*, Jun. 50 (6): 1351-6, 2012.
- 9- DURHAM B.H., JOSEPH F., BAILEY L.M. and FRASER WD.: The association of circulating ferritin with serum concentrations of fibroblast growth factor-23 measured by three commercial assays. *Ann. Clin. Biochem.*, Sep. 44 (Pt 5): 463-6, 2007.
- 10- 3607 Parkway Lane, Suite 100, Norcross, GA 30092. Web: [www.RayBiotech.com](http://www.RayBiotech.com), Email: [info@raybiotech.com](mailto:info@raybiotech.com)
- 11- HU M.C., SHIIZAKI K., KURO-O M., et al.: Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu. Rev. Physiol.*, 75: 503-533, 2013.
- 12- RAMON I., KLEYNEN P., BODY J.J., et al.: Fibroblast growth factor 23 and its role in phosphate homeostasis. *Europ J. Endocrinol.*, 162: 1-10, 2010.
- 13- VOGIATZI M.G., MACKLIN E.A., FUNG E.B., et al.: Bone disease in thalassemia: A frequent and still unresolved problem. *J. Bone Miner Res.*, 24 (3): 543-557, 2009.
- 14- BATRA J., BUTTAR R.S., KAUR P., KREIMERMAN J. and MELAMED M.L.: FGF-23 and cardiovascular disease: Review of literature. *Curr. Opin. Endocrinol. Diabetes Obes.* Dec. 23 (6): 423-429, 2016.
- 15- PRATS M., FONT R., GARCIA C., et al.: Effect of ferric carboxymaltose on serum phosphate and C-terminal FGF23 levels in non-dialysis chronic kidney disease patients: Post-hoc analysis of a prospective study. *BMC Nephrol.*, 14: 167, 2013.
- 16- HARDY S. and VANDEMERGEL X.: Intravenous iron administration and hypophosphatemia in clinical practice. *Int. J. Rheumatol.*, 2015: 1-6, 2015.
- 17- SCHAEFER B., WÜRTINGER P., FINKENSTEDT A., et al.: Choice of high-dose intravenous iron preparation determines hypophosphatemia risk. *PLoS One*, 11: e0167146, 2016.
- 18- HO P.J., TAY L., LINDEMAN R., et al.: Australian Guidelines for the assessment of iron overload and iron chelation in transfusion-dependent thalassemia major, sickle-cell disease and other congenital anemias. *Intern Med. J.*, 41: 516-524, 2011.
- 19- HONDA H., MICHIHATA T., SHISHIDO K., TAKAHASHI K., TAKAHASHI G., HOSAKA N., IKEDA M., SANADA D. and SHIBATA T.: High fibroblast growth factor 23 levels are associated with decreased ferritin levels and increased intravenous iron doses in hemodialysis patients. *PLoS One*, May 5; 12 (5): e0176984, 2017.
- 20- STEFANOPOULOS D., NASIRI-ANSARI N., DONTAS I., VRYONIDOU A., GALANOS A., PSARIDI L., et al.: Fibroblast Growth Factor 23 (FGF23) and Klotho Protein in Beta-Thalassemia. *Horm. Metab. Res.*, 52 (3): 194-201, 2020.
- 21- YANG W.P., CHANG H.H., LI H.Y., LAI Y.C., HUANG T.Y., TSAI K.S., et al.: Iron Overload Associated Endocrine Dysfunction Leading to Lower Bone Mineral Density in Thalassemia Major. *J. Clin. Endocrinol. Metab.*, 105 (4): 5-9, 2020.
- 22- YOSHIKO Y., WANG H., MINAMIZAKI T., IJUIN C., YAMAMOTO R., SUEMUNE S., KOZAI K., TANNE K., AUBIN J.E. and MAEDA N.: Mineralized Tissue Cells Are a Principal Source of FGF23. *Bone*, 40: 1565-1573, 2007.
- 23- RAYEGO-MATEOS S., DOLADÉ N., GARCÍA-CARRASCO A., DIAZ-TOCADOS J.M., IBARZ M. and VALDIVIELSO J.M.: The Increase in FGF23 Induced by Calcium Is Partially Dependent on Vitamin D Signaling. *Nutrients*, 14: 2576, 2022.

## عامل نمو الخلايا الليفية وعلاقته بمستوى الكالسيوم، الفوسفور، والفيريتين في الدم في مرضى أنيميا البحر المتوسط بيتا-ثلاسيميا : دراسة أحادية المركز

يعانى الكثيرون من مرضى أنيميا البحر المتوسط (الثلاسيميا) من مضاعفات كثيرة منها إصابة العظام وتلفها .

يؤثر عامل نمو الخلايا الليفية في الدم (FGF23) في تطور مشاكل العظام المرتبط بالثلاسيميا .

يعتبر ارتباط عامل نمو الخلايا الليفية (FGF23) مع مستوى الكالسيوم والفوسفور والفيريتين في الدم في مرضى الثلاسيميا خطوة مهمة للتحقيق في مشاكل العظام المرتبط بالثلاسيميا وعلاجها . وتهدف هذه الدراسة إلى تقييم مستوى FGF23 في الدم في مرضى الثلاسيميا البالغين وعلاقته بمستويات الفيريتين والكالسيوم والفوسفور .

وقد اشتملت هذه الدراسة على ثلاثة وخمسون مريضاً يعانون من ثلاسيميا بيتا (المعتمدة والغير معتمدة على نقل الدم) وتم استبعاد أى مريض مصاب بالتهاب مزمن أو قصور كلوى وتم تحليل نسبة الفيريتين والفوسفور والكالسيوم وFGF23.

وجد أن متوسط عمر المرضى يتراوح بين (7.6±3.0) سنة بواقع (37.3٪) ذكور و (62.3٪) إناث، (41.5٪) معتمد على نقل الدم و(58.5٪) غير معتمد على نقل الدم. (54.7٪) يعانون من آلام العظام و(18.9٪) يعانون من مضاعفات قلبية.

ووجد ارتباط إيجابى بين FGF23 وكل من آلام العظام ومضاعفات القلب ( $p > 0.001$ )، وارتباط سلبى بين FGF-23 وكل من الفيريتين والكالسيوم ( $p > 0.001$ )، بالإضافة إلى ذلك فإن مستوى FGF23 ومستوى الفوسفور لهما علاقة إيجابية قوية ( $p > 0.001$ ).

وعلى ذلك تم استنتاج أن هناك ارتباط بين FGF-23 ومستويات (الفيريتين، كالسيوم، والفوسفور)، مما يشير إلى مشاركته في الحفاظ على توازن مستوى الكالسيوم كما أن له دور مهم في حدوث مشاكل العظام المرتبطة بالثلاسيميا .