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ORIGINAL RESEARCH **PERIOPERATIVE MEDICINE**

Evaluation of alpha Klotho and fibroblast growth factor-23 levels in Iraqi patients with Graves' disease

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ABSTRACT

Background & Objective: Graves' disease (GD) is an autoimmune condition that targets the thyroid gland, resulting in excessive stimulation and synthesis of thyroid hormones. Excessive synthesis of these hormones can lead to symptoms such as loss of body weight, accelerated heart rate, irritability, reduced tolerance to heat, and excessive perspiration. Alpha-Klotho (KL), a protein crucial for physiological processes, is also involved in GD. The defective immunological condition of GD patients may increase Klotho expression, and the enhanced expression of fibroblast growth factor-23 (FGF23) in GD may be linked to the disease pathophysiology.

We investigated serum levels of KL and FGF23 in Iraqi patients with Graves' disease, analyzing correlations with clinical status and evaluating their potential as biomarkers for disease activity.

Methodology: We conducted this cross-sectional study at The National Diabetes Center, Mustansiriyah University, between December 2022 and April 2023. The study involved 103 patients diagnosed with GD, and 103 patients, aged 21-70 y, as a control group. Patients with multinodular goiter, single thyroid nodules, thyroiditis, pregnant women, and those on medications or oral contraceptives, were excluded.

Result: clinical significance of FGF23 and Klotho (KL) levels in patients with Graves' disease (GD) compared to a control group. Results indicate that the median of KL levels are significantly higher in GD patients (KL = 6.31) compared to the control group (KL = 2.40), with a highly significant difference ($P < 0.001$). In contrast, differences in median of FGF23 levels between GD patients (149) and controls (86.05) were not statistically significant ($P = 0.07$). Furthermore, KL levels were significantly higher in hyperthyroid patients compared to other thyroid statuses ($P = 0.023$), while FGF23 levels did not significantly differ across thyroid statuses ($P = 0.255$). Additionally, the study found a strong correlation between TRAb levels and both KL ($r = 0.291$, $P < 0.001$) and FGF23 ($r = 0.211$, $P = 0.003$), and between KL and FGF23 directly ($r = 0.412$, $P < 0.001$). These findings suggest that while KL may be a significant biomarker in GD, FGF23 relevance appears limited in this context.

Conclusion: In Graves' Disease patients have significantly higher levels of KL and FGF23 compared to controls, suggesting a distinct pathophysiological role for these biomarkers in mineral homeostasis and thyroid hormone regulation.

Abbreviations: AITD - Autoimmune thyroid diseases; KL - Alpha-Klotho; FGF23 - fibroblast growth factor-23; GD - Graves' disease; HT - Hashimoto's thyroiditis; IGF-1R - Insulin-like Growth Factor-1 Receptor;

Keywords: Autoimmune Thyroid Disorders, Hyperthyroidism, Graves' disease, Alpha-Klotho

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1. INTRODUCTION

Autoimmune thyroid diseases (AITD) are caused by malfunction of immunological systems leading to an immune assault on the thyroid. Among autoimmune illnesses and pathogenic thyroid gland disorders, T-cell-mediated organ-specific autoimmune diseases (AITD) are the most often occurring ones.[1] Hashimoto's thyroiditis (HT) and Graves' disease (GD), two main clinical presentations of AITD, are typified by lymphocytic invasion of the thyroid parenchyma. The individual clinical symptoms of GD and HT are thyrotoxicosis and hypothyroidism. Estimates place AITD at 5% of the population.[2] Although GD, the most prevalent cause of hyperthyroidism, affects approximately 20 to 50 individuals out of every 100,000 annually. Characterized by an autoimmune response that leads to the overstimulation of thyroid cells, GD triggers a cascade of systemic effects due to excess thyroid hormone production.[3] The disease predominantly affects women between the ages of 30 and 60 y, with women being significantly more likely to develop GD compared to men.[4]

The immune system's dysfunction, particularly through the formation of autoantibodies against the thyrotropin receptor, underscores the autoimmune nature of GD, leading to widespread clinical symptoms ranging from thyrotoxicosis to severe metabolic disruptions.[5] Expression of this non-specific antigen on fibroblasts and orbital preadipocytes points to possible TSH receptor expression too.[6] There is also a link between ocular alterations and Thyrotropin Receptor (TSHR)-opposing stimulating antibody concentration. GD patients have greater Insulin-like Growth Factor-1 Receptor (IGF-1R) impregnation in the thyroid gland and circling tissues, and self-antibodies against the receptor activate orbital fibroblasts.[7] Clinical signs correlate with the processes of hyperthyroidism and autoimmune diseases. Since excess thyroid hormones affect many different physiological systems, the signs and symptoms of GD can vary substantially and significantly influence general health.[8] Common symptoms are tremor, heat sensitivity and warmth, weight loss despite consistent eating patterns, anxiety and irritability, goiter, changes in menstrual cycles, and others.[9] Almost all of GD signs and symptoms are caused by the goiter, pretibial myxedema, and Graves' ophthalmopathy, which are the primary and secondary effects of hyperthyroidism (which are caused by the autoimmune processes of the disease).

The primary signs of the ensuing hyperthyroidism include sleeplessness. [10] Within this context, the roles of Alpha Klotho (KL) and Fibroblast Growth Factor-23 (FGF23) in GD have garnered interest due to their critical functions in mineral metabolism and potential implications in thyroid pathophysiology ([11]). KL, which acts as a co-receptor for FGF23, is instrumental in enhancing the affinity of FGF23 for its receptors.[12] FGF23 itself is pivotal in regulating phosphate homeostasis and vitamin D metabolism, processes that are often disrupted in metabolic disorders. Importantly, studies have suggested that the levels of FGF23 are elevated in patients with GD, which may contribute to the altered mineral metabolism observed in these individuals.[13] The regulation of mineral metabolism is a very intricate process that seeks to manage the amounts of calcium (Ca^{++}) and phosphate in the bloodstream by means of the interaction between three hormones and three bodily systems.[14] Parathyroid hormone (PTH), vitamin D, and FGF23 work along with the bones, kidneys, and gut to ensure that there are sufficient quantities of Ca^{++} and phosphorus (P) in the plasma. Ca^{++} and P metabolism are intricately interconnected and rely heavily on each other.[6, 8] Both ions are controlled by 1,25-dihydroxyvitamin-D (1,25(OH)2D) (Calcitriol) and PTH. Therefore, Calcitriol enhances the uptake of Ca^{++} and P in the gut, whereas PTH promotes the release of Ca^{++} from bone and the reabsorption of Ca^{++} in the kidney. FGF23, which is produced by osteoblasts and osteocytes, has been demonstrated to reduce the reabsorption of P in the kidney.[15] FGF23 has a role in maintaining the balance of minerals in the body, particularly in controlling the levels of P in the blood. Glycogen storage disease (GSD) is linked to increased bone remodeling, high levels of phosphates in the blood (hyperphosphatemia), and raised levels of FGF23 in the serum.[16]

KL is an enzyme that is produced by the KL gene in humans. The gene associated with an extended longevity in mice was initially identified in 1997. The klotho protein is divided into three subfamilies: α-klotho, β-klotho, and γ-klotho. α-klotho stimulates the activation of FGF23, while β-klotho stimulates the activation of FGF19 and FGF21.[17] The α-klotho gene is located on chromosome 13 and encodes a transmembrane protein with a single-pass integral structure. The α-klotho protein has a brief intracellular region consisting of 11 amino acids, whereas its extracellular portion is lengthy and made up of 980 amino acids.[18] α -Klotho acts as a coreceptor for the phosphatonin FGF23 with the FGF receptor. Additional pleiotropic functions of tissue-Klotho have been identified, including protection against oxidative stress, inhibition of apoptosis and fibrogenesis, promotion of angiogenesis and vascularization, vasculoprotective properties, and regulation of stem cell proliferation, all of which may protect against aging.[18, 19] Strong expression of α-Klotho in thyroid follicular cells suggests that it may be involved in thyroid hormone production regulation.[20] The elevated expression of

Klotho in GD may be caused by the dysfunctional immunological status of GD patients, which may have a protective role, while the increased expression of FGF23 in GD may be related to the disease's pathogenesis.[21]

We investigated Alpha Klotho and FGF23 serum levels in Iraqi Graves' disease patients, analyzing correlations with clinical status and evaluating their potential as biomarkers for the disease activity.

Table 1: Comparison of Kl and FGF23 between the groups

Characteristic	Patient Group (*n* = 103)	Control Group (*n* = 103)	P-value
KL (pg/mL)			
Median (IQR)	6.31 (9.72)	2.40 (6.47)	< 0.001 M ***
Range	0.07-565.21	0.03-10.18	
FGF23 (pg/mL)			
Median (IQR)	149.00 (246.87)	86.05 (69.46)	0.077 M NS
Range	7.36-2475.3	4.01-169.46	

*M: Mann-Whitney U test; NS: not significant; ***: significant at P ≤ 0.01*

2. METHODOLOGY

The National Diabetes Center at Mustansiriyah University, Baghdad, Iraq, conducted a cross-sectional study between December 2022 and April 2023. The study included 103 healthy adults as a control group, whose ages ranged from 21 to 70 y. The study involved 103 patients diagnosed with Graves' disease (GD) by a consultant endocrinologist, including all thyrotoxic patients attending the center during the study period, regardless of their metabolic control status. While some patients were under metabolic control on antithyroid drugs, others remained thyrotoxic at the time of recruitment. The exclusion criteria was patients with multinodular goiter, single thyroid nodules, thyroiditis, pregnant women, and those on medications that could affect the metabolic hormone panel, such as oral contraceptives.

2.1. Sample collection

A volume of 5 ml of venous blood was collected from the veins on the back of the hand or in the area in front of the elbow. The blood was then put in a tube with clotting agent and allowed to coagulate for 20 min. Next, perform centrifugation at a speed of 2000–3000 RPM for a duration of 10 min. The sera were subsequently divided into two Eppendorf tubes for instant testing.

Determination of KL and FGF23

The HumaReader HS measures using the ELISA concept. The testing basis of these kits is the quantitative sandwich enzyme immunoassay method. Precoated onto a microplate is an antibody specific to the antigen. A standard pipette was used to pour samples into wells; the immobilized antibody binds any antigen found there. The wells were filled with a biotin-conjugated antibody specific for antigen following the removal of any unbound molecules. Following washing, the wells were filled with avidin-conjugated horseradish peroxidase (HRP). After a wash to eliminate any unbound avidin-enzyme reagent, a substrate solution is introduced to the wells and color develops in line with the initial step's antigen bound concentration. The color development pauses, and the color intensity is assessed.

3. RESULTS

The current investigation discovered that increased FGF23 levels in GD is not clinically significant. The research methodology, sample size, and particular parameters under investigation may be the cause of this. FGF23 might have a variable clinical value in other illnesses because the importance of biomarkers in a particular medical condition can vary. Table 1 show that KL reached 6.31 pg/mL in GD patients, but it drops down to 2.4 in the control group, and the difference is highly significant (P < 0.001). however; FGF 23 was found to be 149 in GD patients and 86.05 in the control group and the difference is not statistically significant (P = 0.07), but numerically in favor of GD patients.

At the time of enrollment 56.3% are euthyroid being on antithyroid drugs, while the remining 39.8% are hypothyroid, however only 3.9% iatrogenic hypothyroidism being overtreated antithyroid drugs as shown in Figure 1.

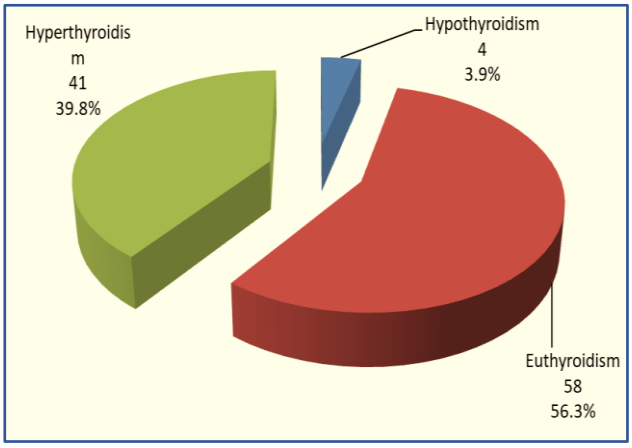

Figure 1: A pie chart illustrates the frequency distribution of individuals with thyroid illness based on their thyroid condition.

Table 2: KL and FGF23 in GD patients with and without proptosis

Characteristics	Proptosis (n = 67)	No Proptosis (n = 36)	P-value
KL (pg/mL)			
Median (IQR)	5.80 (8.79)	6.49 (10.57)	0.855 M
Range	0.16-20.00	0.07-20.00	NS
FGF23 (pg/mL)			
Median (IQR)	152.47 (238.74)	64.51 (277.40)	0.961 M
Range	7.36-375.23	8.01-375.23	NS

M: Mann Whitney U test; NS: not significant

Table 3: Correlation Coefficients among TRAb, KL, and FGF23 in a Biomedical Study

	TRAb		KL	
	r	p	r	P-value
FGF23	0.211	0.003 **	0.412	< 0.001***
KL	0.291	< 0.001 ***	---	---

*** significant at P ≤ 0.01; *** significant at P ≤ 0.001*

Comparison of KL and FGF23 is shown in Table 2. There was no significant difference in KL level and in FGF23 level (P > 0.05). Table 2 shows that in GD patients the presence of proptosis had no impact on KL and FGF23 levels (P = 0.855), (P = 961) respectively.

Table 3 presents the correlation coefficients between (TRAb), Klotho (KL), and (FGF23) by Pearson correlation coefficient. The correlation coefficient (r) and P-values are provided to statistically evaluate the relationships. TRAb shows a positive correlation with both FGF23 and KL, indicated by r-values of 0.211 and 0.291, respectively, with statistically significant P = 0.003 and P < 0.001. KL also exhibits a strong correlation with FGF23, with an r-value of 0.412 and a P < 0.001.

Comparison of KL and FGF23 in thyroid function categories is shown in Table 4. KL was significantly higher in hyperthyroid status (P = 0.023), but there was no significant difference in FGF23 level according to thyroid status (P = 0.255).

4. DISCUSSION

The present study investigated the serum levels of KL and FGF23 in a sample of Iraqi patients with GD and compared them to a control group. The results showed that both KL and FGF23 levels were significantly higher in GD patients compared to healthy controls. Increased concentration of KL in thyroid follicular cells of GD patients suggests that this protein may be regulated during thyroid hormone synthesis.[22] The defective immune status of GD patients may contribute to the enhanced expression of KL. Similarly, the elevated FGF23 levels observed in GD patients may be related to the pathophysiology of the disease. FGF23 is known to play a role in mineral and phosphate homeostasis, and dysregulation of this pathway has been implicated in various thyroid disorders, FGF23 plays a crucial role in maintaining mineral balance by regulating phosphate homeostasis and vitamin D metabolism. It lowers kidney phosphate reabsorption and blocks vitamin D activation, regulating calcium and phosphate intake. Dysregulation of FGF23 in thyroid disorders leads to abnormal mineral metabolism, especially in conditions like GD, where elevated levels disrupt phosphate and calcium regulation.[14,23] The study found that KL and FGF23 levels were highest in hyperthyroid GD patients and lowest in hypothyroid patients, with euthyroid patients showing intermediate values. This pattern likely reflects the dynamic changes in thyroid hormone status over the course of the disease. Interestingly, the presence of

Table 4: Comparison of KL and FGF23 Levels Across Thyroid Function Categories

Parameters	Hypothyroid (n = 4)	Euthyroid (n = 58)	Hyperthyroid (n = 41)	p
KL (pg/mL)				
Median (IQR)	0.21 (6.46)	4.63 (7.92)	7.79 (10.41)	0.023 O *
Range	0.10-8.66	0.07-20.00	0.08-20.00	
FGF23 (pg/mL)				
Median (IQR)	17.11 (170.03)	144.81 (249.07)	161.37 (260.74)	0.255 O NS
Range	8.06-229.16	7.36-375.23	7.70-375.23	

O: One-Way ANOVA; NS: not significant

ophthalmopathy did not seem to impact KL or FGF23 levels. Importantly, the study demonstrated a positive correlation between thyroid-stimulating hormone receptor antibody (TRAb) titers and both KL and FGF23 levels. This suggests that these biomarkers may be useful in monitoring disease activity and severity in GD patients. [24]

5. CONCLUSION

The study found that patients with Graves' disease have significantly higher levels of KL and FGF23 compared to controls, suggesting a distinct pathophysiological role for these biomarkers in mineral homeostasis and thyroid hormone regulation. This study enhances our understanding of Graves' disease biomarkers and sets a foundation for future research on their mechanistic roles and therapeutic intervention targets.

6. Data availability

The numerical data generated during this research is available with the authors.

7. Acknowledgement

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8. Financial support and sponsorship:

This was a self-funded study.

9. Ethical considerations

The research was selected by the Scientific Committee of the Medical Chemistry Department / College of Medicine / University of Al-Qadisiyah, and the research was approved by the Council of the College of Medicine in 17/11/2022.

11. Conflicts of interest:

The authors declare that they have no competing interests and no conflicts of interest.

12. Authors contribution

MFR: literature search, statistical analysis

HAA, AMR: literature search, statistical analysis, manuscript editing

REFERENCES

1. Kahaly GJ, Frommer L, Schuppan D. Celiac disease and endocrine autoimmunity–the genetic link. Autoimmun Rev. 2018;17(12):1169-75. [PubMed] DOI: 10.1016/j.autrev.2018.05.013
2. Salah RB, Kacem FH, Soomauro S, Chouaib S, Frikha F, Charfi N, et al. Autoimmune thyroiditis associated with autoimmune diseases. Electron J Gen Med. 2022;19(6). DOI: 10.29333/ejgm/12399
3. Taylor PN, Albrecht D, Scholz A, Gutierrez-Buey G, Lazarus JH, Dayan CM, et al. Global epidemiology of hyperthyroidism and hypothyroidism. Nat Rev Endocrinol. 2018;14(5):301-16. [PubMed] DOI: 10.1038/nrendo.2018.18
4. Vanderpump MP. Epidemiology of thyroid disorders. The thyroid and its diseases: Springer; 2019. p. 75-85.
5. Ek M, Roth B, Nilsson PM, Ohlsson B. Characteristics of endometriosis: A case-cohort study showing elevated IgG titers against the TSH receptor (TRAb) and mental comorbidity. Eur J Obstet Gynecol Reprod Biol. 2018;231:8. [PubMed] DOI: 10.1016/j.ejogrb.2018.09.034
6. Neag E, Smith T. 2021 update on thyroid-associated ophthalmopathy. J Endocrinol Invest. 2022;45(2):235-59. [PubMed] DOI: 10.1007/s40618-021-01663-9
7. Marín-Sánchez A, Álvarez-Sierra D, González O, Lucas-Martin A, Sellés-Sánchez A, Rudilla F, et al. Regulation of TSHR expression in the thyroid and thymus may contribute to TSHR tolerance failure in Grave's disease patients via two distinct mechanisms. Front Immunol. 2019;10:1695. [PubMed] DOI: 10.3389/fimmu.2019.01695
8. Khan MS, Lone SS, Faiz S, Farooq I, Majid S. Graves' Disease: Pathophysiology, Genetics and Management. In: Graves' Disease. IntechOpen; 2021. DOI: 10.5772/intechopen.98238
9. Antonelli A, Fallahi P, Rotondi M, Ferrari SM, Romagnani P, Grosso M, et al. Increased serum CXCL10 in Grave's disease or autoimmune thyroiditis is not associated with hyper-or hypothyroidism per se, but is specifically sustained by the autoimmune, inflammatory process. Eur J Endocrinol. 2006;154(5):651-8. [PubMed] DOI: 10.1530/eje.1.02137
10. Pokhrel B, Bhusal K. Graves Disease. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2024.
11. Antonelli A, Fallahi P, Elia G, Ragusa F, Paparo SR, Ruffilli I, et al. Grave's disease: Clinical manifestations, immune pathogenesis (cytokines and chemokines) and therapy. Best Pract Res Clin Endocrinol Metab. 2020;34(1):101388. [PubMed] DOI: 10.1016/j.beem.2020.101388
12. Mace ML, Olgaard K, Lewin E. New aspects of the kidney in the regulation of fibroblast growth factor 23 (FGF23) and mineral homeostasis. Int J Mol Sci. 2020;21(22):8810. [PubMed] DOI: 10.3390/ijms21228810
13. Erben RG. Physiological actions of fibroblast growth factor-23. Front Endocrinol. 2018:267. [PubMed] DOI: 10.3389/fendo.2018.00267
14. Agoro R, Ni P, Noonan ML, White KE. Osteocytic FGF23 and its kidney function. Front Endocrinol. 2020;11:592. [PubMed] DOI: 10.3389/fendo.2020.00592
15. Rayego-Mateos S, Doladé N, García-Carrasco A, Diaz-Tocados JM, Ibarz M, Valdivielso JM. The increase in FGF23 induced by calcium is partially dependent on vitamin D signaling. Nutrients. 2022;14(13):2576. [PubMed] DOI: 10.3390/nu14132576
16. Goltzman D. Approach to Hypercalcemia. In: Feingold KR, Anawalt B, Blackman MR, et al., editors. Endotext. South Dartmouth (MA): MDText.com, Inc.; 2000. Available from: https://www.ncbi.nlm.nih.gov/books/NBK279129/

17. Lin CH, Chang CK, Shih CW, Li HY, Chen KY, Yang WS, et al. Serum fibroblast growth factor 23 and mineral metabolism in patients with euthyroid Grave's diseases: A case-control study. Osteoporos Int. 2019;30(11):2289-97. [PubMed] DOI: 10.1007/s00198-019-05116-1

18. Huang C-L, Moe OW. Soluble α-klotho—a fibroblast growth factor 23–independent hormone. Fibroblast Growth Factor 23: Elsevier; 2021. p. 233-40.

19. Hanson K, Fisher K, Hooper NM. Exploiting the neuroprotective effects of α-klotho to tackle ageing-and neurodegeneration-related cognitive dysfunction. Neuronal Signal. 2021;5(2). [PubMed] DOI: 10.1042/NS20200101

20. Bellido T, Pajevic PD, Bonewald L. Osteocyte Biology. Genetics of Bone Biology and Skeletal Disease: Elsevier; 2018. p. 227-40.

21. Pawlikowski M, Pisarek H, Borkowska M, Winczyk K. Expression of α-Klotho protein in human thyroid cancers—an immunohistochemical study. Endokrynol Pol. 2019;70(3):237-40. [PubMed] DOI: 10.5603/EP.a2019.0004

22. Wang W, Su Y, Xu J, Yao W, Qin Q, He S, et al. Elevated serum concentrations of FGF23 and Klotho in patients with Grave's disease and their significance. Clin Med China. 2018:481-4. DOI: 10.3760/cma.j.issn.1008-6315.2018.06.001

23. Kuro-o M. Klotho, phosphate and FGF23 in ageing and disturbed mineral metabolism. Nat Rev Nephrol. 2013;9(11):650-60. [PubMed] DOI: 10.1038/nrneph.2013.111

24. Yuan Q, Shen F, Chen B, et al. FGF23 as a new biomarker for differentiating Graves' disease and Hashimoto's thyroiditis. J Endocrinol Invest. 2018;41(2):149-57.

25. Kadooh QA, Al-Ziaydi AG, Hamza AJ. Evaluation of the influence of trastuzumab therapy on serum levels of HER-2 protein and breast cancer cell lines. Menopause Rev. 2023;23(2):57-63. DOI: 10.5114/pm.2024.139607

