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ORIGINAL ARTICLES

Correlation between the ratio of fibroblast growth factor 21 to klotho with the incidence and severity of diabetic retinopathy in type 2 diabetes mellitus patients

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ABSTRACT

Background and aim: Diabetes Mellitus (DM) is a chronic metabolic illness characterized by high blood sugar levels resulting from reduced insulin secretion, action, or both. Diabetic retinopathy affects 50 to 60% of individuals with DM, leading to blindness in 2.6% of cases. In diabetic retinopathy, FGF21 levels increase while Klotho levels decrease, resulting in a higher ratio of FGF21 to serum Klotho levels.

Methods: In Makassar, this cross-sectional investigation was carried out at the Hasanuddin University Teaching Hospital and the Wahidin Sudirohusodo Hospital from December 2023 until January 2024. The study included type 2 DM patients aged over 18 years. The analysis used descriptive methods and statistical tests, considering results significant if the p-value was <0.05.

Results: Eighty-eight diabetic individuals, ages 52.41±11.32 on average, were enrolled in the study. Of them, 24 participants (27.3%) had non-proliferative diabetic retinopathy (NPDR), 25 subjects (28.4%) had proliferative diabetic retinopathy (PDR), and 39 subjects (44.3%) had no retinopathy. The rate and severity of retinopathy caused by diabetes were significantly correlated with elevated blood FGF21 levels (p=0.005). According to the rate and degree of diabetic retinopathy, 4.1 (p=0.009; OR 3.24, CI 95% 1.3-8.01) was determined to be the ideal cut-off point for the ratio of serum FGF21 levels to serum Klotho levels.

Conclusions. This study discovered a correlation between a high ratio of serum FGF21 levels to serum Klotho levels and the incidence and severity of type 2 DM patients' diabetic retinopathy.

Keywords: diabetic retinopathy, FGF21, Klotho, type 2 DM

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic illness characterized by high blood sugar levels resulting from reduced insulin secretion, action, or both [1]. As of 2019, approximately 463 million adults aged 20 to 79 worldwide were diagnosed with DM, comprising 9.3% of the adult population worldwide. By 2030, this number likely increase to 10.2% of the world's adult population (578 million adults). Indonesia is expected to have 8.4 million type 2 DM cases in 2000 compared to 21.3 million cases by 2030, according to the World Health Organization (WHO) [2,3].

One of the most typical microvascular issues and the main reason behind vision impairment in people of working age worldwide is diabetic retinopathy. It is estimated that 50 to 60% of type 2 DM patients develop diabetic retinopathy, with 2.6% of them progressing to blindness. Clinically, diabetic retinopathy is characterized by retinal neovascularization, microaneurysms, and protein exudates in the vitreous, all contributing to vision impairment [4,5]. The high incidence of vision-threatening diabetic retinopathy (VTDR) and both recognized and undiagnosed diabetic retinopathy underscores the urgent need for comprehensive eye care services and regular screening for DM patients [4,5].

Multiple researches have revealed that increased amounts of Fibroblast Growth Factor (FGF) 21 correspond to the degree of diabetic retinopathy, as reported by Jin *et al.* (2021) [6], Lin *et al.* (2014) [7] and Wang *et al.* (2019) [8]. FGF21, a liver-produced endocrine subfamily comprising 210 amino acids, is vital in regulating blood sugar, lipid metabolism, and atherosclerosis [9,10].

Klotho is primarily produced in the kidneys, with smaller amounts found in the brain's choroid plexus, parathyroid glands, and retinal layer. Klotho comes in three varieties: α, β, and γ. The physiological effects of FGF21 are mediated when this hormone binds to β-Klotho as a co-receptor. Reduced kidney function, indicative of decreased renal mass, leads to lower Klotho production. Additionally, Klotho levels are influenced by oxidative stress and inflammation. FGF and Klotho's endocrine subfamily regulate phosphate, glucose, fatty acid metabolism, energy balance, sympathetic activity, and circadian rhythms [11,12].

Several studies, including those by Ji *et al.* (2020) [13] and Corcillo *et al.* (2020) [14], have reported that in patients with type 2 DM, lower Klotho levels are related to an increased risk of diabetic retinopathy. Klotho is an anti-inflammatory agent in the retina, preventing oxidative stress, inhibiting neovascularization, and reducing endothelial dysfunction [12]. Consequently, diabetic retinopathy is characterized by increased FGF21 levels and decreased Klotho levels, leading to an elevated ratio of FGF21 to Klotho serum levels. The objective of this investigation is to assess the relationship between the ratio of FGF21 to Klotho and the occurrence and severity of diabetic retinopathy in patients with type 2 diabetes.

METHODS

In Makassar, this cross-sectional investigation was carried out at the Hasanuddin University Teaching Hospital and the Wahidin Sudirohusodo Hospital from December 2023 until the required sample size was achieved. The study population comprised type 2 DM patients treated at the Endocrine Metabolic Clinic of both hospitals. The research sample was drawn from type 2 DM patients who were at least 18 years old, willing to engage in the study, and signed the informed consent form as the inclusion criteria. The exclusion criteria included patients with retinal disorders other than diabetic retinopathy, a history of glaucoma, a history of eye nerve surgery, thyroid disorders, and those who were uncooperative.

The dependent variable in this study was the degree of retinopathy, identified among proliferative and non-proliferative diabetic retinopathy. The retinopathy examination involved pupil dilation using 1% tropicamide (and 2.5% phenylephrine if necessary), followed by a 30-minute wait until both pupils were fully dilated. Two examiners then conducted the examination, though not necessarily consecutively. The degree of diabetic retinopathy was assessed using fundus photographs taken 30 minutes after dilation, capturing one 45° image centered on the fovea. Using an ELISA kit (Elabsciense®), the independent variables, FGF21 and Klotho levels, were assessed.

Data analysis comprised both descriptive and statistical methods, utilizing the Statistical Package for the Social Sciences (SPSS) version 25. If the *p*-value for a statistical test was less than 0.05, the results were considered significant. Findings were presented narratively, supported by tables and figures.

[33]The Biomedical Research Ethics Committee on Humans at the Faculty of Medicine, Hasanuddin University, Makassar, granted ethical approval for the study (approved number 848/[23]UN4.6.4.5.31/PP36/2023).

RESULTS

The study sample consisted of 88 subjects, including 25 males (28.4%) and 63 females (71.6%), aged 18-65, [5]with a mean age of 52.41±11.32 years. The subjects' body mass index (BMI) ranged from 17.7 to 34.5 kg/m², with a mean BMI of 24.79±3.70 kg/m². HbA1c levels were ≥7% in 79 subjects (89.8%) and <7% in 9 subjects (10.2%). Among the subjects (Table 1), 39 (44.3%) had no retinopathy, 24 (27.3%) had NPDR, and 25 (28.4%) had PDR.

Table 1. Research subjects' characteristics

Variable	N	%
[31]Gender		
Male	25	28.4
Female	63	71.6
Age		
≥ 60 years	29	32.9
< 60 years	59	67.1
BMI*		
Obese	41	46.6
Non-obese	47	53.4
[3]Diabetic Retinopathy		
Non-Diabetic Retinopathy	39	44.3
Non-Proliferative Diabetic Retinopathy	24	27.3
Proliferative Diabetic Retinopathy	25	28.4
HbA1C level		
≥7 %	79	89.8
<7 %	9	10.2

*BMI: Body Mass Index

The characteristics of variables in research subjects with diabetic retinopathy are detailed in Table 2. Table 2 demonstrated a significant difference in the mean age and creatinine levels between participants with and without diabetic retinopathy, suggesting a correlation between these variables and the incidence of the condition. Meanwhile, the mean BMI of both groups, those without retinopathy and those with retinopathy, fell into the overweight and obese categories, with the mean BMI being lower in subjects with retinopathy compared to those without. Both groups, with and without retinopathy, demonstrated suboptimal blood sugar control, as evidenced by higher mean HbA1c levels in subjects with retinopathy than those without. However, this disparity was not statistically significant. Consequently, in this study, it was concluded that HbA1c was not correlated with the incidence of diabetic retinopathy.

Subjects with diabetic retinopathy had considerably greater mean serum FGF21 levels than subjects without the condition. On the other hand, although this difference was negligible, those with diabetic retinopathy had mean serum Klotho levels that were lower than those without.

Table 2. Characteristics of variables in research subjects with diabetic retinopathy

Variable	Retinopathy Category		
	Non-Diabetic Retinopathy (n = 39)	**Diabetic Retinopathy** (n = 49)	***p*-value***
Age	49.46 ± 12.56	54.76 ± 9.73	**0.034**
Creatinine	0.786 ± 0.25	1.14 ± 0.61	**0.000**
HbA1c	9.654 ± 1.96	10.00 ± 2.92	0.527
BMI**	25.213 ± 3.76	23.94 ± 4.82	0.170
FGF*** 21	887.57 ± 6.20	1488.31 ± 12.58	**0.005**
Klotho	313.53 ± 3.86	201.46 ± 2.17	0.111

*Statistical analysis using T-test

**BMI: Body Mass Index

***FGF: Fibroblast growth factor

The relationship between the elevation of serum FGF21 levels and the prevalence and severity of retinopathy caused by diabetes is shown in Table 3. Notably, the data revealed a progressive elevation in serum FGF21 levels among subjects with PDR, NPDR, and those without retinopathy, indicating statistically significant differences (p=0.005).

Table 3. Relationship between the incidence and severity of diabetic retinopathy and increased blood FGF21 levels

Variable	Retinopathy Category			*p-value**
	Non-Diabetic Retinopathy (n = 39)	Non-Proliferative Diabetic Retinopathy (n = 24)	Proliferative Diabetic Retinopathy (n = 25)	
FGF** 21 Levels (pg/ml)	8.87 ± 6.2	10.98 ± 10.3	18.62 ± 13.5	0.005

*Statistical analysis using one-way ANOVA

**FGF: Fibroblast growth factor

The receiver operating characteristic (ROC) curve was used to identify the ideal cut-off point of serum FGF21 levels for the incidence of diabetic retinopathy, as shown in Figure 1. It was found that, with a sensitivity of 65.3% and specificity of 51%, the ideal cut-off value for blood FGF21 levels in relation to the occurrence of diabetic retinopathy was 7.44 pg/ml.

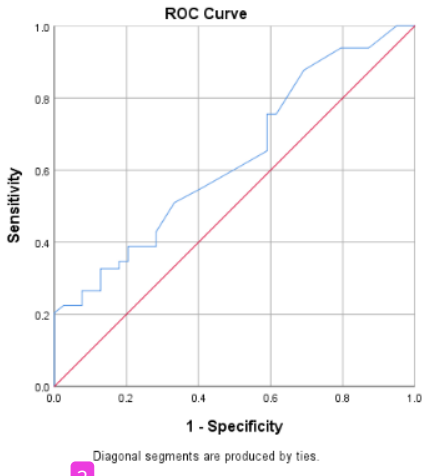

Figure 1. ROC curve between the incidence of diabetic retinopathy with serum FGF21 levels

Table 4 illustrates the relationship between the optimal cut-off point of serum FGF21 levels and the incidence and severity of diabetic retinopathy. It was observed that the optimal cut-off point for serum FGF21 levels concerning the incidence and severity of diabetic retinopathy was 7.44 pg/ml (p=0.542; OR 1.309, 95% CI 0.55-3.11). This result suggested that subjects with serum FGF21 levels ≥7.44 pg/ml were at 1.3 times higher risk for diabetic retinopathy than those with serum FGF21 levels <7.44 pg/ml.

Table 4. Association between optimal serum fgf21 cut-off point and the incidence and severity of diabetic retinopathy

FGF** 21 Levels (pg/ml)	Retinopathy Category		*p*-value*	OR	95% CI
	Non-Diabetic Retinopathy (n = 39)	Diabetic Retinopathy (n = 49)			
≥ 7.44	23 (58.9%)	32 (65.3%)	0.542	1.309	0.55-3.11
<7.44	16 (41.1%)	17(34.7%)			

*Statistical analysis using Chi-Square

**FGF: Fibroblast growth factor

The association between the incidence and severity of diabetic retinopathy and the drop in serum Klotho levels is displayed in Table 5. Although the difference was not statistically significant (p=0.944), it shows that participants with PDR, NPDR, and those without retinopathy had progressively decreased serum Klotho levels.

Table 5. Correlation of serum Klotho reduction with incidence and severity of diabetic retinopathy

Variable	Retinopathy Category			*p-value**
	Non-Diabetic Retinopathy (n = 39)	Non-Proliferative Diabetic Retinopathy (n = 24)	Proliferative Diabetic Retinopathy (n = 25)	
Klotho Levels (ng/ml)	3.13 ± 3.85	2.56 ± 2.84	1.49 ± 1.03	0.944

*Statistical analysis using one-way ANOVA

Table 6 illustrates the correlation between the ratio of serum FGF21 levels to serum Klotho levels and the occurrence and severity of diabetic retinopathy. It reveals a significant association between these ratios, suggesting that a higher ratio of serum FGF21 levels to serum Klotho levels is linked to a greater incidence and severity of diabetic retinopathy.

Table 6. Correlation between the ratio of serum FGF21 to Klotho levels and incidence and severity of diabetic retinopathy

Variable	Retinopathy Category			*p-value**
	Non-Diabetic Retinopathy (n = 39)	Non-Proliferative Diabetic Retinopathy (n = 24)	Proliferative Diabetic Retinopathy (n = 25)	
FGF**21 to Klotho Ratio	3.66	4.60	11.40	0.001

*Statistical analysis using one-way ANOVA

**FGF: Fibroblast growth factor

Figure 2 presents the optimal cut-off point for the serum FGF21 to Klotho ratio concerning the incidence and degree of diabetic retinopathy, as assessed by the ROC curve. It indicates that the optimal cut-off point for this ratio was 4.1, with a sensitivity of 75.5% and specificity of 51.3%.

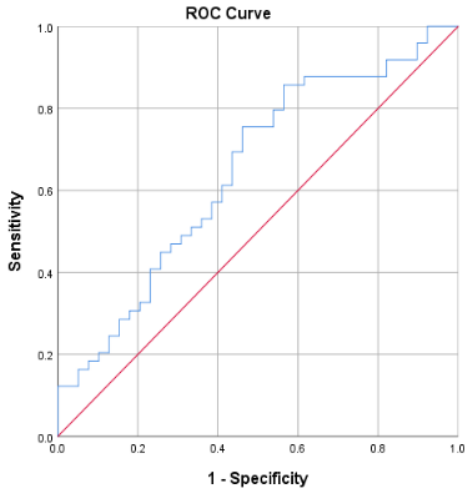

Figure 2. ROC curve of the FGF21 to Klotho ratio concerning the incidence and severity of diabetic retinopathy

Table 7 illustrates the association between the optimal cut-off point of the serum FGF21 to Klotho ratio and the diabetic retinopathy's incidence and severity. The table shows that the optimal cut-off point of this ratio concerning diabetic retinopathy was ≥4.1 (p=0.009; OR 3.24, 95% CI 1.3-8.01). This result suggests that subjects with a serum FGF21 to Klotho ratio ≥4.1 had a 3.24 times increased chance of diabetic retinopathy development than those with a ratio <4.1.

Table 7. Association between the optimal cut-off point of serum FGF21 to serum Klotho ratio and the incidence and severity of diabetic retinopathy

FGF**21 to Klotho Ratio	Retinopathy Category		*p*-value*	OR	95% CI
	Non-Diabetic Retinopathy (n = 39)	**Diabetic Retinopathy** (n = 49)			
<4.1	20 (51.3%)	12 (24.5%)	0.009	3.24	1.31-8.01
≥4.1	19 (48.7%)	37(75.5%)			

*Statistical analysis using Chi-Square
**FGF: Fibroblast growth factor

DISCUSSION

The incidence and severity of diabetic retinopathy showed a significant connection (p=0.005) with elevated blood FGF21 levels in this investigation, suggesting that higher serum FGF21 levels were linked to more severe diabetic retinopathy.

Jin *et al.* (2021) [6], in their study involving 654 type 2 DM subjects, identified a relationship between serum FGF21 levels and the severity of diabetic retinopathy. Research conducted by Heidari and Hasanpour (2021) [15], which included 91 subjects of type 2 DM with diabetic retinopathy, 93 subjects without diabetic retinopathy, and 86 healthy subjects, revealed a significant link between elevated FGF21 levels in all three groups and the severity of diabetic retinopathy (p<0.001). Similarly, a study by Mousavi *et al.* (2017) [16], involving 61 subjects, comprising 25 subjects of type 2 DM with diabetic retinopathy, 22 subjects without diabetic retinopathy, and 14 healthy subjects as controls, found a noteworthy association between increased FGF21 levels and the severity of diabetic retinopathy in all three groups (p=0.003).

FGF21 was identified as an adipokine primarily produced in the liver, functioning as a metabolic hormone involved in lipid and glucose metabolism, insulin resistance, and obesity. Paradoxically, increased FGF21 levels were noted in conditions such as obesity, hypertension, insulin resistance, and metabolic syndrome. This elevation in FGF21 levels in such a condition was interpreted as a compensatory mechanism due to disrupted

metabolic regulation. Moreover, macrovascular and microvascular complications of diabetes were associated with increased FGF21 levels [17-19].

This study also determined the optimal cut-off point for serum FGF21 levels concerning diabetic retinopathy using the ROC curve, assuming that higher serum FGF21 levels correlated with a higher incidence of diabetic retinopathy. The identified cut-off point for serum FGF21 levels regarding the occurrence of diabetic retinopathy was 7.44 pg/ml, with a sensitivity of 65.3% and specificity of 51% (p=0.542; OR 1.309, 95% CI 0.55-3.11), indicating that subjects with serum FGF21 levels ≥7.44 pg/ml were 1.3 times more likely to have developed diabetic retinopathy compared to those with FGF21 levels <7.44 pg/ml.

The research conducted by Esteghamati *et al.* (2016) [20] indicated that serum FGF21 levels above 135.5 pg/ml were correlated with diabetic-related retinopathy. Lin *et al.* (2014) [7] found that serum FGF21 levels exceeding 550 pg/ml were linked to the occurrence of diabetic retinopathy. Similarly, Jin *et al.* (2021) [6] identified a cut-off point of 478.76 pg/ml for serum FGF21 levels, correlating with the rate of diabetic retinopathy.

In this study, consecutive decreases in serum Klotho levels were observed among subjects with PDR, NPDR, and those without diabetic retinopathy. However, these differences were not significant (p=0.944). Ji *et al.* [13] conducted a study in 2020 involving 77 subjects, comprising 60 type 2 DM subjects and 17 healthy subjects as controls. The DM subjects were divided into three subgroups: 27 subjects without diabetic retinopathy, 17 subjects with NPDR, and 16 subjects with PDR. The study found lower serum Klotho levels in diabetic subjects compared to healthy subjects (p=0.007). However, no significant differences were observed between the group without retinopathy and the NPDR group (p=0.425), nor between the NPDR and PDR groups (p=0.123). Corcillo *et al.* (2020) [14], in a study involving 81 subjects, concluded that serum Klotho levels were adversely correlated with a higher risk of diabetic retinopathy.

Klotho is found in the retina, optic nerve, and human lens. It regulated homeostasis mechanisms and retinal cell functions, including phagocytosis, vascular endothelial growth factor A (VEGF-A) secretion, calcium homeostasis, maintenance of oxidative reduction status, and melanin biosynthesis. Klotho significantly reduced VEGF-A, the main pro-angiogenic factor in choroidal neovascularization [21,22].

The lack of correlation between the serum Klotho levels and the incidence and severity of diabetic retinopathy in this study may be because serum Klotho levels were subject to change over time. Therefore, it was anticipated that the results might vary with different examination times.

Furthermore, this study revealed a correlation between increased serum FGF21 to Klotho ratio and the incidence and severity of diabetic retinopathy (p=0.001). This result

suggested that higher serum FGF21 to Klotho ratios were associated with a greater incidence and severity of diabetic retinopathy.

In subjects with diabetic retinopathy, FGF21 levels increased linearly with the severity of retinopathy. Elevated levels of FGF21 have been linked to both the frequency and severity of diabetic retinopathy [6-8]. Conversely, Klotho levels decreased with the severity of diabetic retinopathy. The decrease in Klotho levels was associated with the severity of diabetic retinopathy [13,14]. When there was an increase in serum FGF21 levels and a decrease in serum Klotho levels, the FGF21 to Klotho ratio increased, and the risk of diabetic retinopathy also increased.

The ROC curve was utilized in this investigation to establish the ideal cut-off point for the ratio of the serum FGF21 to the serum Klotho levels with the diabetic retinopathy's incidence and severity. The optimal cut-off point for the ratio of serum FGF21 to Klotho levels to the incidence and severity of diabetic retinopathy was found to be 4.1 with a sensitivity of 75.5% and specificity of 51.3% (p=0.009; OR 3.24, 95% CI 1.3-8.01). This meant that subjects with a serum FGF21 to Klotho ratio ≥4.1 were at a 3.24 times higher risk of developing diabetic retinopathy compared to those with a ratio <4. This indicated that the higher the serum FGF21 level and the lower the serum Klotho level, the greater the diabetic retinopathy's incidence and severity. Using the FGF21 to Klotho ratio also yielded a higher significance level than using either examination alone.

This study had limitations as the duration since patients started suffering from diabetes was unknown, and the research method employed was cross-sectional. Hence, further research using a cohort method was deemed necessary.

CONCLUSION

This study shows that the diabetic retinopathy's incidence and severity in patients with type 2 DM are significantly correlated with higher blood FGF21 to Klotho ratio. In people with type 2 DM, the ratio of serum FGF21 to Klotho shows promise as a predictor of the development and severity of diabetic retinopathy. These findings underscore the potential utility of this ratio as a prognostic marker in diabetic retinopathy assessment and management, offering valuable insights for further research and clinical applications in diabetic patient care.

Conflict of interest: No conflict of interest

Financial disclosure or funding: None to declare

Informed consent:

All subjects participating in a research study involving human participants must sign an informed consent statement before participating. This process ensures that participants are fully aware of the critical elements of the study, including the potential risks and benefits, and that they are making an informed decision about their involvement.

ethics committee approval:

The Faculty of Medicine, Hasanuddin University, in Makassar, South of Sulawesi, Indonesia, has an Ethics Committee for Biomedical Research on Humans and approved this study. Based on recommendation letter Number: 848/UN4.6.4.5.31/ PP36/ 2023, Dec 2023, with protocol number: UH23100754

Author's contributions:

The research concept and design were contributed to by SB, HR, FHP, and HU. AAZ participated in the statistical analysis of the data. All authors prepared the draft, revised the paper, and assessed the content. Each author has reviewed the manuscript, given their approval, and confirmed to the accuracy and and integrity of every finding.

REFERENCES

1. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2014;37:81-90.
2. Indonesian Ministry of Health. Infodatin remains productive and prevents and treats Diabetes Mellitus in 2020. *Indonesian Ministry of Health Data and Information Center*. Published online 2020:1-10.
3. Saeedi P, Petersohn I, Salpea P, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Research and Clinical Practice*. 2019;157.
4. Sinclair SH, Schwartz SS. Diabetic Retinopathy–An Underdiagnosed and Undertreated Inflammatory, Neuro-Vascular Complication of Diabetes. *Front Endocrinol*. 2019;10:1-14.
5. Ansari P, Tabasumma N, Snigdha NN, et al. Diabetic Retinopathy: An Overview on Mechanisms, Pathophysiology and Pharmacotherapy. *Diabetology*. 2022;3(1):159-175.
6. Jin S, Xia N, Han L. Association between serum fibroblast growth factor 21 levels and sight-threatening diabetic retinopathy in Chinese patients with type 2 diabetes. *BMJ Open Diabetes Research and Care*. 2021;9(1).
7. Lin Y, Xiao YC, Zhu H, et al. Serum fibroblast growth factor 21 levels are correlated with the severity of diabetic retinopathy. *Journal of Diabetes Research*. 2014.
8. Wang YS, Ye J, Cao YH, et al. Increased serum/plasma fibroblast growth factor 21 in type 2 diabetes mellitus: A systematic review and meta-analysis. *Postgraduate Medical Journal*. 2019:134-139.
9. Violetta L, Kartasasmita AS, Supriyadi R, Rita C. Circulating Biomarkers to Predict Diabetic Retinopathy in Patients with Diabetic Kidney Disease. Published online 2023:1-13.
10. Deng J, Liu Y, Liu Y, Li W, Nie X. The multiple roles of fibroblast growth factor in diabetic nephropathy. *Journal of Inflammation Research*. 2021;14:5273 -5290.
11. Wong CW, Wong TY, Cheng CY, Sabanayagam C. Kidney, and eye diseases: Common risk factors, etiological mechanisms, and pathways. *Kidney International*. 2014;85(6):1290-1302.
12. Tang A, Zhang Y, Wu L, et al. Klotho's impact on diabetic nephropathy and its emerging connection to diabetic retinopathy. *Frontiers in Endocrinology*. 2023;14:1-17.
13. Ji B, Wei H, Ding Y, et al. Protective potential of klotho protein on diabetic

retinopathy: Evidence from clinical and in vitro studies. *Journal of Diabetes Investigation*. 2020;11(1):162-169.

14. Corcillo A, Fountoulakis N, Sohal A, Farrow F, Ayis S, Karalliedde J. Low levels of circulating anti-aging hormone Klotho predict the onset and progression of diabetic retinopathy. *Diabetes and Vascular Disease Research*. 2020;17(6).
15. Heidari Z, Hasanpour M. The serum fibroblast growth factor 21 is correlated with retinopathy in patients with type 2 diabetes mellitus. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*. 2021;15.
16. Mousavi Z, Bonakdaran S, Sahebkar A, et al. The relationship between serum levels of fibroblast growth factor 21 and diabetic retinopathy. *Excli Journal*. 2017;16:1249 -1256.
17. Farooq M, Khan AW, Kim MS CS. The Role of Fibroblast Growth Factor (FGF) Signaling in Tissue Repair and Regeneration. *Cells*. 2021;10(3242):3-20.
18. Tomita Y, Fu Z, Wang Z, et al. Long-acting FGF21 inhibits retinal vascular leakage in in vivo and in vitro models. *International Journal of Molecular Sciences*. 2020;21(4).
19. Fisher FM, Maratos-Flier E. Understanding the Physiology of FGF21. *Annual Review of Physiology*. 2016;78:223 -241.
20. Esteghamati A, Momeni A, Abdollahi A, et al. Serum fibroblast growth factor 21 concentrations in type 2 diabetic retinopathy patients. *Annales d'Endocrinologie* . 2016;77(5):586-592.
21. Puddu A, Maggi DC. Klotho: A new therapeutic target in diabetic retinopathy? *World Journal of Diabetes*. 2023;14(7):1027-1036.
22. Hua S, Liu Q, Li J, Fan M, Yan K, Ye D. Beta-klotho in type 2 diabetes mellitus: From pathophysiology to therapeutic strategies. *Reviews in Endocrine and Metabolic Disorders*. 2021;22(4):1091-1109.