The association between fibroblast growth factor 21 with diabetes retinopathy among type 2 diabetes mellitus patients: a systematic review, meta-analysis, and meta-regression

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Background. Diabetic retinopathy (DR), a leading cause of vision loss worldwide, is a common complication of type 2 diabetes mellitus (T2DM) driven by chronic hyperglycemia and microvascular damage. Fibroblast growth factor 21 (FGF21) is crucial in blood sugar regulation and has been linked to DR incidence and severity. While some studies suggest that FGF21 levels may contribute to the DR incidence, others propose a protective role. This discrepancy necessitates further analysis, prompting this study to evaluate the association between FGF21 levels and DR incidence and severity in T2DM patients.

Methods: A systematic search was conducted through MEDLINE, Web of Science, Scopus, and Embase up to May 2024 for studies evaluating the association between FGF21 and DR incidence and severity. A random-effect model meta-analysis was performed to calculate the pooled standardized mean difference (SMD) and 95% confidence intervals (CI). A univariate meta-regression was performed to analyze factors influencing pooled size estimates. All statistical analyses were performed using STATA 17 software.

Result: This systematic review and meta-analysis of 5852 participants revealed that FGF21 was positively correlated with DR (SMD 3.11; 95% CI 0.92 to 5.30, p = 0.005) and sight-threatening DR (STDR) incidence (SMD 3.61; 95% CI 0.82 to 6.41, p = 0.01). There was no significant difference in FGF21 levels in DR vs STDR (p = 0.79). Subgroup analysis revealed a significant difference in DR incidence between LDL groups, with higher DR incidence in the group with low-density lipoprotein (LDL) levels >100 (P < 0.00001). Meta-regression revealed no variables significantly influenced the pooled size estimates.

Conclusion: A higher level of FGF21 was associated with higher DR and STDR incidence among T2DM patients, highlighting its potential utilization as a biomarker for DR detection and enabling the exploration of FGF21-based treatment strategies. However, variables independently predicting DR among patients with elevated FGF21 levels shall be explored further.**PROSPERO ID.** CRD42024559142.

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Abstract

Background. Diabetic retinopathy (DR), ~~is~~ a leading cause of vision loss worldwide, is a common complication of type 2 diabetes mellitus (T2DM) driven by chronic hyperglycemia and microvascular damage. Fibroblast growth factor 21 (~~FGF~~FGF21)~~-21)~~ is crucial in blood sugar regulation and has been ~~, in which have reported to be correlated~~linked ~~with~~ to DR incidence and severity. ~~However~~While some studies suggest that FGF21 levels may contribute to the DR incidence, others propose a protective role. This discrepancy necessitates further analysis, prompting ~~, outcomes of previous studies remains contentious. Hence,~~ this study ~~aims~~ to evaluate the association between ~~FGF~~FGF21~~-21~~ levels ~~to~~ and DR incidence and severity ~~among~~ in ~~T2DM~~ T2DM patients.

Methods: A systematic search was conducted through MEDLINE, Web of Science, Scopus, and Embase up to May 2024 for studies evaluating the association between ~~FGF-21~~FGF21 and DR incidence and severity. A random-effect model meta-analysis was performed to calculate the pooled standardized mean difference (SMD) and 95%

confidence intervals (CI). A univariate meta-regression was performed to analyze factors influencing pooled size estimates. All statistical analyses were performed using STATA 17 software.

Result: This systematic review and meta-analysis of ~~A total of~~ 5852 participants revealed that ~~from seven studies were included.~~ ~~FGF-21~~FGF21 was positively correlated with DR ~~incidence~~ (SMD 3.11; 95% CI 0.92 to 5.30, p = 0.005) and sight-threatening DR (STDR) incidence (SMD 3.61; 95% CI 0.82 to 6.41, p = 0.01). There was no significant difference in FGF21 levels in DR vs ~~sight-threaten~~STing DR (p = 0.79). Subgroup analysis revealed a significant difference in DR incidence between LDL groups, with higher DR incidence in the group with low-density lipoprotein (LDL) levels >100 (P < 0.00001). Meta-regression revealed no variables significantly influenced the pooled size estimates.

Conclusion: A higher level of ~~FGF-21~~FGF21 was associated with higher DR and STDR incidence among T2DM patients, highlighting its ~~.~~potential utilization as a biomarker for DR detection and enabling the exploration of FGF21-based treatment strategies. However, variables independently predicting DR among patients with elevated ~~FGF-21~~FGF21 levels shall be explored further.

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Introduction

Diabetes Mellitus (DM) is a chronic metabolic disorder characterized by elevated blood sugar levels arising from defects in insulin secretion, insulin action, or both.[1,2] Prolonged elevation of blood sugar levels can lead to damage and dysfunction of several specific organs, such as the eyes, kidneys, nerves, heart, and blood vessels. Various epidemiological studies indicate a tendency towards increased incidence and prevalence of ~~type 2 DM~~type 2 diabetes mellitus (T2DM) worldwide. [3] In 2019, the estimated number of adults aged 20 to 79 years suffering from DM was 463 million, accounting for 9.3% of the total adult population globally. [4] This number is projected to reach 578 million, or approximately 10.2% of the total adult population worldwide in 2030. [5]

T2DM is known as the most prevalent type of DM, accounting for over 90% of all diabetes cases worldwide. Characterized by a progressive loss of insulin secretion due to insulin

resistance, T2DM is known as the leading cause of death and disability worldwide, mainly due to diabetes-related complications. ~~Chronic~~ Cc~~c~~omplications of DM can manifest as vascular disturbances in both microvascular and macrovascular systems and dysfunction in the nervous system or neuropathy. [1]

Macrovascular complications, such as those ~~commonly~~ affecting the ~~organs such as the~~ heart, brain, and blood vessels, are significant concerns in diabetes management. However, ~~while~~ microvascular complications~~,~~ particularly ~~occur in organs such as the eyes and kidneys.~~ [5,6] ~~d~~Diabetic retinopathy (DR), pose a substantial burden among ~~is a major microvascular complication and a leading cause of vision impairment in~~ the working-age population worldwide. Not only that, it contributes as the leading cause of visual impairment worldwide; 50~~ to~~ 60% of ~~type 2 DM~~T2DM patients are estimated to experienc~~e~~ing DR ~~complications~~, and 2.6% of them have vision loss. [3,7]

DR is broadly classified into two categories: nonproliferative diabetic retinopathy, described as DR, and advanced proliferative or sight-threatening DR (PDR/STDR)[8]. The two categories were differentiated by the presence of pathologic retinal neovascularization, which is known to be a hallmark of STDR. This pathological condition arises from chronic hyperglycemia-induced vascular damage, mediated through the activation of the polyol pathway, the accumulation of advanced glycation end products (AGEs), the protein kinase C (PKC) pathway and the hexosamine pathway, all of which contribute to oxidative stress.[9] These processes lead to the loss of pericyte, a defining hallmark of DR. The loss of pericytes, which provides structural support, results in capillary walls outpouching, further contributing to microaneurysm formation[10].

As these processes advance, hemorrhages from this newly formed aneurysm may present and impair vision [10]. This condition ~~due to diabetic retinopathy~~ can ~~affect~~ severely decrease the quality of life and productivity, especially among working-age groups. ~~, impacting the economic conditions of affected individuals and their surroundings.~~ Notably, among those who experienced DR,~~The main challenge in managing diabetic retinopathy is~~ early-stage DR is often asymptomatic, resulting in

delayed diagnosis and treatment initiation. This delay contributes to the high~~, as most patients in the early stages do not present with visual complaints. The high~~ prevalence of undiagnosed ~~diabetic retinopathy~~DR and ~~vision~~sight-threatening ~~diabetic retinopathy~~DR (S~~V~~TDR), underscor~~inges~~ the urgent need for ~~evaluating~~ comprehensive ~~the extent of~~ eye health services, including regular vision screening for individuals with DM ~~for DM patients, especially through screening~~.[11]

In addition to traditional biomarkers such as HbA1c, microalbuminuria, and urine albumin creatinine ratio (UACR), various molecular biomarker assays have emerged for predicting and assessing the incidence of ~~diabetic retinopathy~~DR. These biomarkers are associated with mechanisms involved in the occurrence of ~~diabetic retinopathy~~DR such as hypoxia, oxidative stress, inflammation, endothelial dysfunction, and angiogenesis~~.~~ .[6]~~Chronic hyperglycemia leads to the accumulation of advanced glycation end products (AGEs), activation of protein kinase C (PKC), dysregulation of polyol pathways, and hexosamine activation, all of which trigger oxidative stress resulting in thickening of the basement membrane, retinal ischemia, increased VEGF, and neovascularization causing non-proliferative and proliferative diabetic retinopathy.~~[12] ~~On another pathway, pericyte damage, endothelial dysfunction, damage to the blood-retinal barrier (BRB), and increased vascular permeability lead to macular edema.~~ [11]

Human fibroblast growth factor (FGF) ~~consists of 22 groups generally divided into three subfamilies: paracrine subfamily, endocrine subfamily, and non-signal FGF subfamily~~comprises 22 groups generally divided into three subfamilies: paracrine, endocrine, and non-signal FGF. [13] Fibroblast growth factor 21 (~~FGF 21~~FGF21), a member of the endocrine subfamily, is produced in the liver and is a 210-amino acid polypeptide that plays a crucial role in atherosclerosis, blood sugar regulation, and lipid metabolism.[14,15]

FGF21 is expressed in response to stress triggers like oxidative stress from reactive oxygen species (ROS), and it has been found to interact with a high-affinity receptor called β-klotho, which functions as a single-pass transmembrane protein.[16] Interestingly,

FGF21 has complex relationships with T2DM, which initially plays a protective role in the early phase of DM by improving glucose homeostasis through reducing β-cells apoptosis and dysfunction via PPARδ/γ signaling pathways. [17] However, increased concentrations of FGF21 are paradoxically observed in heightened insulin resistance, obesity, diabetes, and metabolic syndrome. Despite its unclear paradoxical increase mechanisms, this phenomenon is suggested as the result of resistance and compensatory response to a dysregulated metabolic state. [18–20]

~~Several~~ Despite several studies that reported a significant correlation between increased FGF21 levels and the ~~severity~~ incidence of ~~diabetic retinopathy~~DR~~, as reported by Shi Jin et al., Yuan Lin, and Yun-Sheng Wang.~~[14,21,22]~~.~~, another study revealed no significant association between FGF21 levels and risk of DR incidence within five years.[23] Moreover, another study demonstrated that FGF21 levels are not significantly associated with DR incidence.[24] Hence, these~~However, previous studies have~~ controversial conclusions regarding the significance of ~~FGF-21~~FGF21 as a predicting biomarker for DR's incidence and severity warrant further analysis. ~~Moreover~~In addition, comprehensive analysis of independent factors predicting DR incidence remains ~~contentious~~varied. Therefore, this study aims to analyze the ~~pooled estimates regarding the~~ association between serum ~~FGF-21~~FGF21 levels and the incidence and severity of DR ~~among~~ in T2DM patients. U~~ts.~~nderstanding the association between FGF21 levels will potentially enable its use as a biomarker for DR detection and allow targeted therapeutic strategies in T2DM and DR management.

Materials & Methods

This systematic review and meta-analysis was conducted per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 guidelines[25]. This review has been registered on PROSPERO under the number CRD42024559142. The protocol is described as follows.

a. Search strategy

A systematic search was conducted on MEDLINE, Web of Science, Scopus, and EMBASE with coverage up to April 2024 was performed initially using the following

keywords: “fibroblast growth factor 21” AND “diabetic retinopathy,” and their synonyms, which combined using Boolean operators. Complete details of the search strategy are available in **Supplemental Material 2.** The search results from ~~both~~ all databases were exported and imported into Covidence, software for literature screening in systematic reviews. All titles and abstracts from the search were cross-referenced to identify duplicates and any potential missing studies. Titles and abstracts were screened for a subsequent full-text review. Two authors (ASDN and HB) independently performed the complete search strategy. Any disagreements were resolved through discussion with the referee or third author (MA).

b. Inclusion and exclusion criteria

The inclusion criteria for studies were: (1) written in English; (2) evaluating the association of fibroblast growth factor 21 with ~~retinopathy diabetes~~DR incidence and severity; (3) observational study design (cross-sectional study, cohort, or case-control); (4) measured and reported the association between ~~FGF-21~~FGF21 levels and DR incidence/severity in numerical values; and (5) human subjects. Exclusion criteria included: (1) duplicate reports; (2) no full-text available; (3) conference abstracts, review, case reports, case series, and meta-analysis; and (4) studies with insufficient data to extract.

c. Study selection and data extraction

After a review of abstracts, relevant articles were retrieved and reviewed for further analysis. Bibliographies of these articles provided further references. Two independent reviewers (ASDN and HB) reviewed all retrieved records ~~independently~~. Uncertainties were resolved via discussion with a third reviewer (MA). Data extracted from each included study were first author’s name, publication year, study design, country, sample size, age, sex proportion, duration of diabetes, the levels of ~~FGF-21~~FGF21 in T2DM patients without DR, with ~~non sight-threatening~~ DR, and sight-threatening DR (STDR).

d. Data extraction and quality assessment

Relevant papers were thoroughly identified, and their information, such as author, publication year, country, study design, sample size, age, ~~FGF-21~~FGF21 levels, BMI, and HbA1c, were extracted. Two reviewers will independently assess the risk of bias in included studies using the Newcastle–Ottawa Scale (NOS) for observational studies (cohort, case-control, and cross-sectional studies) to assess participant selection, comparability between groups, and ascertainment of exposure or outcome. Ratings ranged from zero to nine, categorizing studies as poor quality (zero), fair quality (three to five), and excellent or high quality (six to nine). A third author was brought in in conflicting assessments to help reach an agreement. [26]

e. Statistical analysis

Meta-analysis was conducted to estimate the pooled effect size of ~~FGF21~~FGF21 levels and its ~~and klotho~~ association with ~~diabetes~~ T2DM and ~~retinopathy~~DR. Descriptive data of the participants' characteristics are reported as mean ± standard deviation (SD)~~(SD)~~. Descriptive analyses and figures of risk of bias were performed using a spreadsheet (Microsoft Excel 2016© USA). In contrast, meta-analytic statistics were calculated using STATA version 17 (Stata Corporation, College Station, TX, USA). The standardized mean difference (SMD), the number of participants, and the standard error of the SMD for each study were used to quantify changes in the performance variables when comparing the level of ~~FGF21~~FGF21 in patients with T2DM without DR, with DR, and with STDR. SMDs for each study group were calculated using Hedges's g.[27] SMDs were weighted by the inverse of variance to calculate an overall effect and its 95% confidence interval (CI). The net treatment effect was obtained by subtracting the SMD of the control group from the SMD of the experimental group. The variance was calculated from both groups' pooled SD of change scores. Subgroup analysis was performed for race, high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol (TC), and triglycerides (TG) levels.

A univariate, random-models meta-regression analysis was performed to investigate whether clinical or laboratory indices could independently predict DR incidence in the pooled analysis. The independent variables examined included HbA1c, age, sex,

race, duration of DM, systolic blood pressure (SBP), LDL cholesterol, HDL cholesterol, TG, and TC levels. For the ~~each~~ dependent variable (~~(~~DR incidence), the effect of each independent variable was tested. The restricted maximum likelihood method is employed under the random effects model. A significant P-value was defined as less than 0.1. The ~~tau$_2$~~ tau^2 and I_2 statistical indices were used to assess heterogeneity.

To avoid problems using Q statistics to assess ~~systematic differences (~~heterogeneity~~y)~~, ~~we calculated the~~ I_2 statistics was calculated, indicating the percentage of observed total variation across studies due to absolute heterogeneity rather than chance. I_2 interpretation is intuitive and lies between 0% and 100%. An I_2 value between 25% and 50% represents a small amount of inconsistency, an I_2 value between 50% and 75% represents a medium amount of heterogeneity, and an I_2 value >75% represents a large amount of heterogeneity. A restrictive categorization of values for I_2 would not be appropriate for all circumstances, although it would tentatively accept adjectives of low, moderate, and high to I_2 values of 25%, 50%, and 75%, respectively.[28]

f. Sensitivity analysis

Sensitivity analysis was used to determine whether any single study or group of studies significantly influenced the overall results. ~~whether any single study or group of studies significantly influenced the overall results.~~ The leave-one-out method eliminated a single study at a time. If substantial heterogeneity occurred, subgroup analysis was employed to find the sources of heterogeneity. The leave-one-out method omits one study at a time and was performed using STATA version 17.

g. Publication bias

Publication bias and small-study effects ~~was~~ were assessed visually using a funnel plot and statistically using Egger's test [29]. The asymmetrical or disproportional distribution data in the funnel plot evidenced the presence of publication bias. ~~were distributed disproportionately.~~ In contrast, the absence of publication bias was suggested when the data were distributed approximately symmetrically. Additionally,

a significant p-value of Egger's test indicates the presence of publication bias and small study effects.[30] Egger's test was calculated using STATA version 17. ~~and significant Egger's test imply a significant publication bias and small study effects.~~

Results

a. Study selection

A total of 248 studies were initially obtained from five databases (MEDLINE, Web of Sciences, EMBASE, ScienceDirect, and Scopus) and manually from the references of included studies~~previous reviews~~. Among them, 127 duplicate records were removed automatically before screening. During the screening process, 83 articles with irrelevant titles/abstracts were excluded, leaving 38 potential ones for further identification. A total of 31 studies were excluded due to unsuitable study design (review/case report/letters to the editor), including other type(s) of diabetes or complication(s), different fibroblast growth factors, irrelevant outcome(s), or unavailable full-text. Seven studies fulfilled the criteria and were then assessed for study quality, and all of them were included in the pooled analysis. The detail of the study flow diagram (PRISMA) can be seen in **Figure 1.**

Figure 1. PRISMA Flow Diagram.

b. Characteristic of included study

Eventually, seven studies were included for qualitative synthesis [31–37] (**Table 1),** and six studies incorporating 5852 participants (710 NDR, 356 with NPDR, and 4786 with STDR) were finally pooled in a meta-analysis. The six included studies published between 2014 and 2021, with the sample size ranging from 47 to 4760. Three studies were conducted in Iran, two in China, and one in South Korea. Five studies compared the mean ~~FGF-21~~FGF21 levels in DR and NDR~~,~~ and four compared the mean ~~FGF-21~~FGF21 levels in NDR and STDR. Additionally, the levels of serum ~~FGF-21~~FGF21 were compared in three included studies. The characteristics of the included study are detailed in Table 1.

Table 1. Characteristics of Included Study

c. Study quality assessment

The quality assessment of each included study was performed using NOS. All six included studies were rated moderate to high quality. The quality assessment of each study using the NOS critical appraisal checklist is listed in Table 1.

d. Association between ~~FGF21~~FGF21 and ~~retinopathy diabetes~~DR incidence

Five studies involving 889 patients reported serum FGF21 levels for DR. The present study demonstrates a significant ~~inverse~~ positive association between ~~FGF-21~~FGF21 levels and DR ~~retinopathy diabetes~~ incidence ~~. It is revealed that with the significant heterogeneity, the lower level of FGF-21 is significantly correlated with DR incidence~~ (SMD 3.11; 95% CI 0.92 to 5.30, p = 0.005) (Figure 2~~Figure 2~~). This indicates that higher levels of FGF21 predict the incidence of DR among T2DM patients.

Figure 2. Association between ~~FGF-21~~FGF21 levels with DR incidence

e. ~~,~~ Association between ~~FGF21~~FGF21 with ~~sight-threatening retinopathy diabetes~~STDR incidence

Further analysis was performed to calculate the association between ~~FGF-21~~FGF21 in T2DM patients without DR and T2DM with STDR. Four pooled studies found a significant positive association between ~~FGF-21~~FGF21 levels and STDR (SMD 3.61; 95% CI 0.82 to 6.41, p = 0.01) (Figure 3). Significant heterogeneity was found in this pooled analysis. ~~It is noticed that larger effect size was found in STDR incidence compared to DR incidence, indicating~~ A larger effect size was found in STDR than in DR incidence, indicating a possible association between ~~FGF-21~~FGF21 levels and DR severity.

Figure 3. Association between ~~FGF-21~~FGF21 levels with STDR incidence

f. Association between ~~FGF-21~~FGF21 levels with DR severity ~~of retinopathy diabetes~~

~~In order to~~Five studies were included in this effect size estimate to evaluate the association between DR severity and serum FGF21 levels ~~evaluate the association between DR severity and serum FGF-21~~FGF21 ~~levels, five studies were included in this effect size estimates~~. However, it ~~is~~ was revealed that the association between

serum ~~FGF-21~~FGF21 levels and DR severity among NPDR and STDR patients was insignificant (p = 0.79) (Figure 4).

Figure 4. Association between ~~FGF-21~~FGF21 levels with DR severity

g. Subgroup analysis

Subgroup analyses investigating the association between serum ~~FGF-21~~FGF21 levels and DR incidence are depicted in **Figure 5**. From the subgroup analysis, larger pooled effect sizes were observed among Asian populations (SMD 4.71; 95% CI 1.33 to 8.10, p = 0.006), individuals with higher HDL levels (>40 mg/dL) (SMD 4.09; 95% CI 1.12 to 7.07, p = 0.007), higher LDL levels (>100 mg/dL) (SMD 6.50; 95% CI 5.28 to 7.71, p < 0.001), and lower TG levels (<130 mg/dL) (SMD 3.78; 95% CI 2.26 to 5.31, p < 0.001).

However, significant differences across all subgroups were only evident in the pooled analysis of LDL levels, where LDL levels >100 mg/dL were significantly associated with higher DR incidence in the pooled samples (SMD 6.50; 95% CI 5.28 to 7.71, p < 0.001). In contrast, no statistically significant differences were observed among subgroups defined by race, HDL, triglyceride (TG), and total cholesterol (TC) levels, as indicated by non-significant tests of subgroup difference.

Figure 5. Summary of ~~subgroup analysis~~Subgroup Analysis

h. Sensitivity analysis

~~Through our sensitivity analysis using the leave-one-out method, we~~Our sensitivity analysis using the leave-one-out method found no significant changes in the pooled estimates of Hedges's g when excluding each study one at a time (Figure 6). Omitting any study from the pooled analysis did not affect the statistical significance of the overall outcomes. This finding ~~indicate~~ indicates that no single study ~~had a substantial impact on~~substantially impacted the overall findings.

Figure 6. Sensitivity Analysis

i. Publication bias

The funnel plot analysis suggested no obvious evidence of publication bias in the pooled estimates, as the plot displayed approximately symmetrical (Figure 7). Regression-based Egger's test analysis revealed insignificant estimates, indicating no small study effects in the pooled analysis (Z=-1.54, p= 0.12).

Figure 7. Funnel plot

j. Meta-regression

Univariable meta-regression analysis ~~was conducted to investigate~~investigated whether ~~any~~ clinical or laboratory indices could independently predict DR incidence in T2DM patients. However, none of the examined clinical indices (including HbA1c, age, sex, race, duration of DM, systolic blood pressure (SBP), LDL cholesterol, HDL cholesterol, and TC levels) were found to be significant independent predictors of DR incidence in this population. Detailed results of the meta-regression are summarized in Table 2.

Table 2. Summary of meta-regression

Discussion

To our knowledge, this study is the first systematic review and meta-analysis to analyze the association of ~~protein FGF-21~~FGF21 ~~with diabetic retinopathy~~DR. ~~Our study demonstrates that higher FGF-21~~FGF21 ~~levels predicts the incidence of DR and STDR among T2DM patients. This study acts as a~~the protein FGF21 with DR. Our study demonstrates that higher FGF21 levels predict the incidence of DR and STDR among T2DM patients. This study provides robust data to further validate the utilization of ~~FGF-21~~FGF21 as a biomarker of DR in T2DM patients.

In chronic hyperglycemia, there is an accumulation of advanced glycation end products (AGEs), activation of protein kinase C (PKC), dysregulation of polyol pathways, and activation of hexosamine pathways. All these factors trigger oxidative stress, leading to basement membrane thickening, retinal ischemia, increased vascular endothelial growth factor (VEGF), and neovascularization, which cause non-proliferative and proliferative ~~diabetic retinopathy~~DR.[38] In other pathways, ~~there is~~

pericyte damage, endothelial dysfunction, blood-retinal barrier (BRB) damage, and increased vascular permeability leadingpericyte damage, endothelial dysfunction, blood-retinal barrier (BRB) damage, and increased vascular permeability lead to macular edema.[11,39]

FGF21FGF21 is an endocrine hormone primarily produced synthesized by the liver and adipose tissue, regulatregulated ed by peroxisome proliferator-activated receptors (PPAR)δα and PPARγ, that plays a role in glucose metabolism, fat, insulin resistance, and obesity..[40–43] Conditions associated with increased oxidative stress, such as hyperglycemia, lead to elevated levels of FGF21, which has complex effects on T2DM.[40,44] Initially, elevated FGF21 levels provide protection againstprotect DM by improving glucose metabolism through several liver-mediated pathways. Firstly, FGF21 reduces insulin resistance by stimualting insulin secretion via the PI3K/ Akt signallingstimulating insulin secretion via the PI3K/ Akt signaling pathway, thereby enhancing postprandial insulin sensitivity.[45,46] Secondly, FGF21 protects pancreatic β-cells by promoting islet autophagy, which is mediated by the activation of AMPK-acetyl coenzyme A carboxylase (ACC) and PPARδ/γ signaling pathways, which contributes to the survival and functionality of β-cell, preventing their dysfunction. [47] Thirdly, FGF21 enhances insulin sensitivity by inhibiting hepatic mTORC1, further elucidating its protective role against DM. [45,47,48] However, this protective effects of FGF21 appears to be most significant in the early stages It functions physiologically to increase gluconeogenesis and ketone body production during starvation and tohyperglycemia, as FGF21 appears to be elevated in chronic hyperglycemia as a compensatory or resistance effectsthe protective effects of FGF21 appear to be most significant in the early stages of hyperglycemia, as FGF21 appears to be elevated in chronic hyperglycemia as a compensatory or resistance effect.[20] enhance insulin sensitivity postprandially.

Interestingly, our study found theaan association between increased FGF21 levels and the incidence and severity of DR. Although the mechanisms linking serum FGF21FGF21 levels to DR are not wellpoorly understood, it is speculated hypothesized that elevated FGF21FGF21 in diabetic complications emerges due to

'FGF21 resistance' or a paradoxical increase. [49] ~~This paradoxical phenomenon occurs alongside dysfunctional or compensatory mechanisms in receptor complex expression as previously noted in animal models~~As previously noted in animal models, This paradox occurs alongside dysfunctional or compensatory mechanisms in receptor complex expression. Consequently, while FGF21 levels may have protective effects ~~againts~~against T2DM in its early phase, chronic hyperglycemia-induced FGF21 resistance is proposed to be the ~~mechanisms~~mechanism explained by the result of our study, ~~may be a compensatory response to metabolic stress and vascular endothelial dysfunction. This mechanism might be analogous to the hyperglycemia-associated resistance to adiponectin,~~ where increased serum ~~FGF21~~FGF21 levels initially aim to repair microvascular damage in retinopathy, serving as a ~~counterative~~counteractive mechanism against metabolic stress and vascular endothelial damage in DR.[11,14,45]

This mechanism occurs similarly in ~~microvascular protecting~~the microvascular protection effect by FGF21. As a regulator of glucose metabolism, FGF21 promotes intrahepatic gluconeogenesis during starvation. However, under hyperglycemic states, FGF21 ~~suppresss~~suppresses glucogenic gene expression, reducing hepatic glucose production and maintaining glucose homeostasis. [47,48]

It is worth noting that administration of FGF21 has been shown ~~to significantly increase plasma adiponectin concentrations~~to increase plasma adiponectin concentrations significantly. Adiponectin, an insulin-sensitizing, anti-inflammatory, anti-atherosclerotic, and hepatoprotective factor predominantly produced from adipocytes, contributes to ~~the amelioration of DR~~DR ameliorating.[47] This finding is further supported by a study by Tomita et al., which demonstrated that administration of (PPAR)δ modulator, known to upregulate FGF21 levels, inhibited pathological angiogenesis in the retina of mouse model by ~~surpressing~~suppressing hypoxia-inducible factor (HIF) and VEGF system.[43,50] Our result suggests that the observed increase of FGF21 levels in DR and STDR, with ~~a more pronounced effects~~a more pronounced effect in STDR, could be explained by two potential mechanisms: 1) a compensatory response to FGF21 resistance as β-cell dysfunction worsens, or 2) an

effort to repair microvascular damage and inhibit pathological neovascularization in DR cases. This mechanism ~~was in line~~aligned with previous findings ~~which explains~~, which explain the U-shaped relationship between FGF-21 and microvascular complications in T2DM [20].

Despite our pooled analysis ~~did not identify~~not identifying any clinical indices as ~~an independent factors of DR incidence, previous studies have highlighted significant associations of obesity (BMI >30), high cholesterol levels, HbA1c, and FGF21~~FGF21 ~~were~~ an independent factor of DR incidence, previous studies have highlighted significant associations of obesity (BMI >30), high cholesterol levels, HbA1c, and FGF21 associated with DR incidence.[31] Another study by Esteghamati et al.~~,~~ revealed that ~~DR incidence among T2DM patients were significantly predicted by FGF-21~~FGF21 ~~levels, duration of DM, and TG levels~~FGF21 levels, duration of DM, and TG levels significantly predicted DR incidence among T2DM patients.[35] Previous studies have identified age, duration of diabetes, hyperglycemia, hypertension, and hyperlipidemia as known risk factors for DR.[51,52]. This association between serum ~~FGF21~~FGF21 and DR has been supported by previous research.[53]

However, it is worth noting that different studies utilized different cut-off values of FGF21 as a biomarker for DR. Jung et al.~~, used cut off values of ≤113, 113-214, and ≥214 as cut off values, with higher values in the latter group reflects~~ used cut-off values of ≤113, 113-214, and ≥214 as cut-off values, with higher values in the latter group reflecting higher risks of DR development.[20] On the other hand, Heidari et al.~~, revealed that FGF-21 predicts~~ predict the incidence of DR with the optimal cut-off value of >312 pg/mL with sensitivity of 97.80 (92.3-99.7) and specificity of 96.77 (90.9-99.3). Both NPDR and PDR were predicted under the ~~model of AUC 0.990~~AUC 0.990 model.[31] In ~~study by Esteghamati et al., a~~ study by Esteghamati et al., the c~~C~~linical cut-off for the pooled samples was 135/5 pg/ml L with a sensitivity of 97.8% and specificity of 75.0%. This study stated that patients with serum ~~FGF21~~FGF21 ≥ 135.5 pg/mL had a 25.86~~135~~-fold increased risk of T2DR.[35] The proposed cutoff of

~~FGF21~~FGF21 to predict T2DR, 135.5 pg/mL, is much lower than that reported by Lin et al.[37]. In Lin et al.~~, [37]study, the mean serum FGF21~~FGF21 levels were 125.9, 326.8, 631.9 and 669.4 pg/ml in controls, T2DM patients without retinopathy, NPT2DR~~ [37]study, the mean serum FGF21 levels were 125.9, 326.8, 631.9, and 669.4 pg/ml in controls, T2DM patients without retinopathy, NPT2DR, and PT2DR patients, respectively, which is higher than that calculated for all groups in Esteghamati et al., study. ~~In other hand, Mousavi et al., found the best cut-off values for FGF21 in T2DM at 196 pg/mL, with the~~On the other hand, Mousavi et al. found the best cut-off values for FGF21 in T2DM at 196 pg/mL, with a sensitivity of 80% and specificity of 47.2%. Hence, the disparities of optimal cut-off values across studies might influence the potential bias within the analysis. Further studies should investigate the optimal cut-off values of FGF21 to validate its clinical utility and enable it as a biomarker in clinical settings.

Ac~~However, ac~~cording to Lin et al., the proposed independent factors of DR were ~~FGF-21~~FGF21, age, diabetes duration, and HDL levels. ~~Whereas~~ On the other hand, independent factors for STDR incidence were ~~FGF-21~~FGF21, age, diabetes duration, and diastolic blood pressure.[37] Lin et al.~~, divided their samples into four-quartiles~~ divided their samples into four quartiles with different levels of ~~FGF-21~~FGF21, with Q1 being the lowest (~~FGF-21~~FGF21 < 388 pg/mL) and Q4 ~~for~~ being the highest (~~FGF-21~~FGF21 ≥~~>~~ 580 pg/mL). From their pooled analysis, the patients in Q4 had a higher prevalence of DR and STDR ($p<0.05$). Serum ~~FGF21~~FGF21 level >478.76 pg/mL suggested the occurrence of DR ~~and that~~, and a level >554.69 pg/mL indicated STDR ($p<0.01$).

The finding of this study is supported by a study by Jin et al., which classified T2DM patients based on their ~~FGF21~~FGF21 serum levels~~, This~~. This found that patients in the highest quartile (Q4) had significantly higher risks of DR and STDR ~~compared to~~than those in the lowest quartile (Q1), even after adjusting for confounding factors. ROC analysis indicated that serum ~~FGF21~~FGF21 levels above 554.69 pg/mL were associated with an over eight-fold increased risk of STDR. [34] These findings align

with our pooled analysis, which demonstrated that higher concentrations of ~~FGF21~~FGF21 predict the severity of DR.

Despite the findings, the mechanisms behind increased serum ~~FGF-21~~FGF21 levels in patients with DR remain unclear. Elevated ~~FGF-21~~FGF21 in these patients may be a compensatory response to metabolic stress, known as ~~FGF-21~~FGF21 resistance. [54] This resistance, characterized by increased circulating ~~FGF-21~~FGF21 and decreased receptor expression, has been associated with a compensatory increase in adiponectin levels in obese individuals, those with insulin resistance, and heart failure patients.[55,56]

~~FGF-21~~FGF21 may target the vascular system, protecting against atherosclerosis by inducing adiponectin to inhibit neointima formation and inflammation~~,~~ and suppressing hepatic cholesterol synthesis to reduce hypercholesterolemia.[57,58] It also promotes angiotensin II metabolism in adipocytes and renal cells, mitigating hypertension and vessel injury.[58] Lei Ying et al. found that ~~FGF-21~~FGF21 improved aortic dilation in diabetes mice via oxidative stress suppression and endothelial nitric oxide synthase activation.[59]

The correlation between ~~FGF-21~~FGF21 and DR incidence resembles hyperglycemia-associated adiponectin resistance. Increased serum ~~FGF-21~~FGF21 may compensate for endothelial dysfunction in retinopathy, with defects in ~~FGF-21~~FGF21 expression or activation reducing insulin sensitivity, liver fatty acid oxidation, and triglyceride clearance.[13,54,55,57] Elevated ~~FGF-21~~FGF21 levels in conditions like metabolic syndrome, obesity, insulin resistance, diabetes, and hypertension suggest a response to poor metabolic status.[60,61]

Understanding the complex relationship between FGF21 and diabetes~~, along with its complications,~~ and its complications has paved the way for novel pharmaceutical strategies to overcome T2DM. Tomita et al.~~,~~ demonstrated that long-acting FGF21

could reduce retinal vascular leakage in mice with retinal disorders.[62] This finding is further supported ~~as a study by Fu et al.,~~ by a study by Fu et al., which revealed that FGF21 administration suppressed ocular neovascularization in mice through adiponectin-mediated pathways.[63] Additionally, FGF21 inhibited pro-inflammatory agents, such as tumor necrosis factor-α (TNF-α), expression but did not alter *Vegfa* expr ~~ession~~expression in neovascular eyes in mice models. Prior studies have also highlighted the beneficial effects of selective PPARα ~~modulator (SPPARMα), such as pemafibrate~~modulators (SPPARMα), such as fenofibrate, in preventing pathological retinal neovascularization by upregulating liver FGF21 levels.[50] These findings suggest that FGF21 could be a therapeutic target for managing pathological vessel growth in DR. Notably, administration of FGF21 has been shown to not only improve the metabolic benefits of insulin sensitivity~~, but also improving lipid profile and obesity, which also reduce~~ but also improve lipid profile and obesity, which also reduces the risks of metabolic syndrome.[48] However, clinical trials investigating FGF21 ~~theraupetic~~therapeutic potential in human DR are still limited.

However, the discrepancies between clinical studies, especially in ~~context of~~ the context of an optimal cut-off value of ~~FGF-21~~FGF21 levels in predicting DR incidence and severity, may stem from differences in participant characteristics, such as age, BMI, duration of diabetes, glycemic control, and laboratory methods in ~~FGF21~~FGF21 measurements.[64] Our main finding indicates that elevated serum ~~FGF21~~FGF21 ~~predict~~ predicts the incidence of DR and STDR among T2DM patients, although the optimal cut-off value remains unclear.

Building on the findings of this study, clinical efforts should be directed ~~towards integrating As measuring serum FGF-21~~FGF21 ~~levels~~toward integrating serum FGF21 level measurement into practice for early detection and management of DR. As the FGF21 measurement is deemed efficient and feasible in community hospitals, our study supports its ~~use~~ utilization for ~~further~~ eye exams in T2DM patients.

Moreover, research should focus on ~~the validation of optimal cut-off values of FGF21 for DR prediction and exploring the clinical trials in human~~validating optimal cut-off values of FGF21 for DR prediction and exploring the clinical trials in humans for FGF21-based treatments for DR.~~s and therapies.~~ The significant association between ~~FGF-21~~FGF21 levels and DR offers clinicians and researchers insight into a novel pathway for future DR treatment, emphasizing its relevance as a biomarker for monitoring and predicting diabetic complications in ~~type 2 diabetes~~T2DM patients.

Conclusions

The serum level of ~~FGF-21~~FGF21 is a predictive marker for the incidence of DR in patients with T2DM and demonstrates a positive correlation with the severity of DR in T2DM patients. Thus, ~~FGF-21~~FGF21 holds potential as a biomarker for ~~both~~ predicting the incidence of DR and determining the prognosis of T2DM. Understanding t~~T~~he link between serum ~~FGF-21~~FGF21 levels and DR suggests a ~~new~~ pathway for future DR treatment by managing pathological neovascularization via ~~inhibition of~~inhibiting pro-inflammatory agents and adiponectin pathways. ~~.~~

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Conflict of interest

The authors declare none of competing interest to report.

References

[1] American Diabetes Association, Diagnosis and Classification of Diabetes Mellitus, Diabetes Care 37 (2014) S81–S90. https://doi.org/10.2337/dc14-S081.

[2] J. Ye, Y. Wu, S. Yang, D. Zhu, F. Chen, J. Chen, X. Ji, K. Hou, The global, regional and national burden of type 2 diabetes mellitus in the past, present and future: a systematic analysis of the Global Burden of Disease Study 2019, Front Endocrinol (Lausanne) 14 (2023). https://doi.org/10.3389/fendo.2023.1192629.

[3] American Diabetes Eassociation, 11. Microvascular Complications and Foot Care: Standards of Medical Care in Diabetes—2021, Diabetes Care 44 (2021) S151–S167. https://doi.org/10.2337/dc21-S011.

[4] P. Saeedi, I. Petersohn, P. Salpea, B. Malanda, S. Karuranga, N. Unwin, S. Colagiuri, L. Guariguata, A.A. Motala, K. Ogurtsova, J.E. Shaw, D. Bright, R. Williams, 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 Res Clin Pract 157 (2019). https://doi.org/10.1016/J.DIABRES.2019.107843.

[5] P. Saeedi, I. Petersohn, P. Salpea, B. Malanda, S. Karuranga, N. Unwin, S. Colagiuri, L. Guariguata, A.A. Motala, K. Ogurtsova, J.E. Shaw, D. Bright, R. Williams, 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 Res Clin Pract 157 (2019) 107843. https://doi.org/10.1016/j.diabres.2019.107843.

[6] S.H. Sinclair, S.S. Schwartz, Diabetic Retinopathy–An Underdiagnosed and Undertreated Inflammatory, Neuro-Vascular Complication of Diabetes, Front Endocrinol (Lausanne) 10 (2019). https://doi.org/10.3389/fendo.2019.00843.

[7] W. Wang, A. Lo, Diabetic Retinopathy: Pathophysiology and Treatments, Int J Mol Sci 19 (2018) 1816. https://doi.org/10.3390/ijms19061816.

[8] E.J. Duh, J.K. Sun, A.W. Stitt, Diabetic retinopathy: current understanding, mechanisms, and treatment strategies, JCI Insight 2 (2017). https://doi.org/10.1172/jci.insight.93751.

[9] M. Brownlee, The Pathobiology of Diabetic Complications, Diabetes 54 (2005) 1615–1625. https://doi.org/10.2337/diabetes.54.6.1615.

[10] W. Wang, A.C.Y. Lo, Diabetic Retinopathy: Pathophysiology and Treatments, Int J Mol Sci 19 (2018) 1816. https://doi.org/10.3390/ijms19061816.

[11] K. Frudd, S. Sivaprasad, R. Raman, S. Krishnakumar, Y.R. Revathy, P. Turowski, Diagnostic circulating biomarkers to detect vision-threatening diabetic retinopathy: Potential screening tool of the future?, Acta Ophthalmol 100 (2022). https://doi.org/10.1111/aos.14954.

[12] A. Praidou, E. Papakonstantinou, S. Androudi, N. Georgiadis, G. Karakiulakis, S. Dimitrakos, Vitreous and serum levels of vascular endothelial growth factor and platelet-derived growth factor and their correlation in patients with non-proliferative diabetic retinopathy and clinically significant macula oedema, Acta Ophthalmol 89 (2011) 248–254. https://doi.org/10.1111/J.1755-3768.2009.01661.X.

[13] A. Kharitonenkov, T.L. Shiyanova, A. Koester, A.M. Ford, R. Micanovic, E.J. Galbreath, G.E. Sandusky, L.J. Hammond, J.S. Moyers, R.A. Owens, J. Gromada, J.T. Brozinick, E.D. Hawkins, V.J. Wroblewski, D.-S. Li, F. Mehrbod, S.R. Jaskunas, A.B. Shanafelt, FGF-21 as a novel metabolic regulator, Journal of Clinical Investigation 115 (2005) 1627–1635. https://doi.org/10.1172/JCI23606.

[14] S. Jin, N. Xia, L. Han, Association between serum fibroblast growth factor 21 level and sight-threatening diabetic retinopathy in Chinese patients with type 2 diabetes, BMJ Open Diabetes Res Care 9 (2021) e002126. https://doi.org/10.1136/bmjdrc-2021-002126.

[15] E. Szczepańska, M. Gietka-Czernel, FGF21: A Novel Regulator of Glucose and Lipid Metabolism and Whole-Body Energy Balance, Hormone and Metabolic Research 54 (2022) 203–211. https://doi.org/10.1055/a-1778-4159.

[16] Y. Ogawa, H. Kurosu, M. Yamamoto, A. Nandi, K.P. Rosenblatt, R. Goetz, A. V. Eliseenkova, M. Mohammadi, M. Kuro-o, βKlotho is required for metabolic activity of fibroblast growth factor 21, Proceedings of the National Academy of Sciences 104 (2007) 7432–7437. https://doi.org/10.1073/pnas.0701600104.

[17] T. Xie, W.Y. So, X.Y. Li, P.S. Leung, Fibroblast growth factor 21 protects against lipotoxicity-induced pancreatic β-cell dysfunction via regulation of AMPK signaling and lipid metabolism, Clin Sci 133 (2019) 2029–2044. https://doi.org/10.1042/CS20190093.
[18] Y.C. Woo, A. Xu, Y. Wang, K.S.L. Lam, Fibroblast Growth Factor 21 as an emerging metabolic regulator: clinical perspectives, Clin Endocrinol (Oxf) 78 (2013) 489–496. https://doi.org/10.1111/cen.12095.
[19] B.M. Cheung, H. Deng, Fibroblast growth factor 21: a promising therapeutic target in obesity-related diseases, Expert Rev Cardiovasc Ther 12 (2014) 659–666. https://doi.org/10.1586/14779072.2014.904745.
[20] C.-H. Jung, S.-H. Jung, B.-Y. Kim, C.-H. Kim, S.-K. Kang, J.-O. Mok, The U-shaped relationship between fibroblast growth factor 21 and microvascular complication in type 2 diabetes mellitus, J Diabetes Complications 31 (2017) 134–140. https://doi.org/10.1016/j.jdiacomp.2016.10.017.
[21] Y. Lin, Y. Xiao, H. Zhu, Q. Xu, L. Qi, Y. Wang, X. Li, M. Zheng, R. Zhong, Y. Zhang, X. Xu, B. Wu, Z. Xu, X. Lu, Serum Fibroblast Growth Factor 21 Levels Are Correlated with the Severity of Diabetic Retinopathy, J Diabetes Res 2014 (2014) 1–6. https://doi.org/10.1155/2014/929756.
[22] Y.-S. Wang, J. Ye, Y.-H. Cao, R. Zhang, Y. Liu, S.-W. Zhang, W. Dai, Q. Zhang, Increased serum/plasma fibroblast growth factor 21 in type 2 diabetes mellitus: a systematic review and meta-analysis, Postgrad Med J 95 (2019) 134–139. https://doi.org/10.1136/postgradmedj-2018-136002.
[23] K.-L. Ong, A.S. Januszewski, R. O'Connell, L. Buizen, A.J. Jenkins, A. Xu, D.R. Sullivan, P.J. Barter, R.S. Scott, M.-R. Taskinen, K.-A. Rye, A.C. Keech, Relationship of fibroblast growth factor 21 with baseline and new on-study microvascular disease in the Fenofibrate Intervention and Event Lowering in Diabetes study, Diabetologia 58 (2015) 2035–2044. https://doi.org/10.1007/s00125-015-3652-2.
[24] Z. Mousavi, S. Bonakdaran, A. Sahebkar, G. Yaghoubi, M.A. Yaghoubi, N. Davoudian, M. Mohebbi, The relationship between serum levels of fibroblast growth factor 21 and diabetic retinopathy., EXCLI J 16 (2017) 1249–1256. https://doi.org/10.17179/excli2017-672.
[25] Higgins JPT, Thomas J, Chandler J, Cumpston MS, Li T, Page MJ, Welch VA, Cochrane Handbook for Systematic Reviews of Interventions Version 6.2, Cochrane, 2021.
[26] A. Stang, Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses, Eur J Epidemiol 25 (2010) 603–605. https://doi.org/10.1007/s10654-010-9491-z.
[27] C.R. Brydges, Effect Size Guidelines, Sample Size Calculations, and Statistical Power in Gerontology, Innov Aging 3 (2019). https://doi.org/10.1093/geroni/igz036.
[28] J.P.T. Higgins, S.G. Thompson, J.J. Deeks, D.G. Altman, Measuring inconsistency in meta-analyses., BMJ 327 (2003) 557–60. https://doi.org/10.1136/bmj.327.7414.557.
[29] J.A.C. Sterne, M. Egger, Funnel plots for detecting bias in meta-analysis, J Clin Epidemiol 54 (2001) 1046–1055. https://doi.org/10.1016/S0895-4356(01)00377-8.
[30] M. Egger, G.D. Smith, M. Schneider, C. Minder, Bias in meta-analysis detected by a simple, graphical test, BMJ 315 (1997) 629–634. https://doi.org/10.1136/bmj.315.7109.629.
[31] Z. Heidari, M. Hasanpour, The serum fibroblast growth factor 21 is correlated with retinopathy in patients with type 2 diabetes mellitus, Diabetes & Metabolic Syndrome:

Clinical Research & Reviews 15 (2021) 102296. https://doi.org/10.1016/j.dsx.2021.102296.

[32] Z. Mousavi, S. Bonakdaran, A. Sahebkar, G. Yaghoubi, M.A. Yaghoubi, N. Davoudian, M. Mohebbi, The relationship between serum levels of fibroblast growth factor 21 and diabetic retinopathy., EXCLI J 16 (2017) 1249–1256. https://doi.org/10.17179/excli2017-672.

[33] C.-H. Jung, S.-H. Jung, B.-Y. Kim, C.-H. Kim, S.-K. Kang, J.-O. Mok, The U-shaped relationship between fibroblast growth factor 21 and microvascular complication in type 2 diabetes mellitus, J Diabetes Complications 31 (2017) 134–140. https://doi.org/10.1016/j.jdiacomp.2016.10.017.

[34] S. Jin, N. Xia, L. Han, Association between serum fibroblast growth factor 21 level and sight-threatening diabetic retinopathy in Chinese patients with type 2 diabetes, BMJ Open Diabetes Res Care 9 (2021) e002126. https://doi.org/10.1136/bmjdrc-2021-002126.

[35] A. Esteghamati, A. Momeni, A. Abdollahi, A. Khandan, M. Afarideh, S. Noshad, M. Nakhjavani, Serum fibroblast growth factor 21 concentrations in type 2 diabetic retinopathy patients, Ann Endocrinol (Paris) 77 (2016) 586–592. https://doi.org/10.1016/j.ando.2016.01.005.

[36] C.-H. Lee, D.T.-W. Lui, C.Y.-Y. Cheung, C.H.-Y. Fong, M.M.-A. Yuen, Y.-C. Woo, W.-S. Chow, I.Y.-H. Wong, A. Xu, K.S.-L. Lam, Circulating AFABP, FGF21, and PEDF Levels as Prognostic Biomarkers of Sight-threatening Diabetic Retinopathy, J Clin Endocrinol Metab 108 (2023) e799–e806. https://doi.org/10.1210/clinem/dgad112.

[37] Y. Lin, Y. Xiao, H. Zhu, Q. Xu, L. Qi, Y. Wang, X. Li, M. Zheng, R. Zhong, Y. Zhang, X. Xu, B. Wu, Z. Xu, X. Lu, Serum Fibroblast Growth Factor 21 Levels Are Correlated with the Severity of Diabetic Retinopathy, J Diabetes Res 2014 (2014) 1–6. https://doi.org/10.1155/2014/929756.

[38] E.J. Duh, J.K. Sun, A.W. Stitt, Diabetic retinopathy: current understanding, mechanisms, and treatment strategies, JCI Insight 2 (2017). https://doi.org/10.1172/jci.insight.93751.

[39] P. Ansari, N. Tabasumma, N.N. Snigdha, N.H. Siam, R.V.N.R.S. Panduru, S. Azam, J.M.A. Hannan, Y.H.A. Abdel-Wahab, Diabetic Retinopathy: An Overview on Mechanisms, Pathophysiology and Pharmacotherapy, Diabetology 3 (2022) 159–175. https://doi.org/10.3390/diabetology3010011.

[40] M.Á. Gómez-Sámano, M. Grajales-Gómez, J.M. Zuarth-Vázquez, Ma.F. Navarro-Flores, M. Martínez-Saavedra, Ó.A. Juárez-León, M.G. Morales-García, V.M. Enríquez-Estrada, F.J. Gómez-Pérez, D. Cuevas-Ramos, Fibroblast growth factor 21 and its novel association with oxidative stress, Redox Biol 11 (2017) 335–341. https://doi.org/10.1016/j.redox.2016.12.024.

[41] F.M. Fisher, E. Maratos-Flier, Understanding the Physiology of FGF21, Annu Rev Physiol 78 (2016) 223–241. https://doi.org/10.1146/annurev-physiol-021115-105339.

[42] D.M. Ornitz, N. Itoh, The Fibroblast Growth Factor signaling pathway, WIREs Developmental Biology 4 (2015) 215–266. https://doi.org/10.1002/wdev.176.

[43] Y. Tomita, D. Lee, K. Tsubota, T. Kurihara, PPARα Agonist Oral Therapy in Diabetic Retinopathy, Biomedicines 8 (2020) 433. https://doi.org/10.3390/biomedicines8100433.

[44] C. Iacobini, M. Vitale, C. Pesce, G. Pugliese, S. Menini, Diabetic Complications and Oxidative Stress: A 20-Year Voyage Back in Time and Back to the Future, Antioxidants 10 (2021) 727. https://doi.org/10.3390/ANTIOX10050727.

[45] C.C. Rusu, S. Racasan, I.M. Kacso, D. Moldovan, A. Potra, D. Tirinescu, C. Budurea, R. Orasan, I.M. Patiu, C.I. Bondor, D. Vladutiu, M.G. Caprioara, The metabolic hormone FGF21 is associated with endothelial dysfunction in hemodialysis patients, Int Urol Nephrol 49 (2017) 517–523. https://doi.org/10.1007/s11255-016-1474-x.

[46] H. Tan, T. Yue, Z. Chen, W. Wu, S. Xu, J. Weng, Targeting FGF21 in cardiovascular and metabolic diseases: from mechanism to medicine, Int J Biol Sci 19 (2023) 66–88. https://doi.org/10.7150/ijbs.73936.

[47] A. Erickson, R. Moreau, The regulation of *FGF21* gene expression by metabolic factors and nutrients, Horm Mol Biol Clin Investig 30 (2017). https://doi.org/10.1515/hmbci-2016-0016.

[48] L. Geng, K.S.L. Lam, A. Xu, The therapeutic potential of FGF21 in metabolic diseases: from bench to clinic, Nat Rev Endocrinol 16 (2020) 654–667. https://doi.org/10.1038/s41574-020-0386-0.

[49] M. Yang, L. Zhang, C. Wang, H. Liu, G. Boden, G. Yang, L. Li, Liraglutide Increases FGF-21 Activity and Insulin Sensitivity in High Fat Diet and Adiponectin Knockdown Induced Insulin Resistance, PLoS One 7 (2012) e48392. https://doi.org/10.1371/journal.pone.0048392.

[50] Y. Tomita, N. Ozawa, Y. Miwa, A. Ishida, M. Ohta, K. Tsubota, T. Kurihara, Pemafibrate Prevents Retinal Pathological Neovascularization by Increasing FGF21 Level in a Murine Oxygen-Induced Retinopathy Model, Int J Mol Sci 20 (2019) 5878. https://doi.org/10.3390/ijms20235878.

[51] C.-H. Lee, D.T.-W. Lui, C.Y.-Y. Cheung, C.H.-Y. Fong, M.M.-A. Yuen, Y.-C. Woo, W.-S. Chow, I.Y.-H. Wong, A. Xu, K.S.-L. Lam, Circulating AFABP, FGF21, and PEDF Levels as Prognostic Biomarkers of Sight-threatening Diabetic Retinopathy, J Clin Endocrinol Metab 108 (2023) e799–e806. https://doi.org/10.1210/clinem/dgad112.

[52] S. Jin, N. Xia, L. Han, Association between serum fibroblast growth factor 21 level and sight-threatening diabetic retinopathy in Chinese patients with type 2 diabetes, BMJ Open Diabetes Res Care 9 (2021) e002126. https://doi.org/10.1136/bmjdrc-2021-002126.

[53] Z. Heidari, M. Hasanpour, The serum fibroblast growth factor 21 is correlated with retinopathy in patients with type 2 diabetes mellitus, Diabetes & Metabolic Syndrome: Clinical Research & Reviews 15 (2021) 102296. https://doi.org/10.1016/j.dsx.2021.102296.

[54] M. Yang, L. Zhang, C. Wang, H. Liu, G. Boden, G. Yang, L. Li, Liraglutide Increases FGF-21 Activity and Insulin Sensitivity in High Fat Diet and Adiponectin Knockdown Induced Insulin Resistance, PLoS One 7 (2012) e48392. https://doi.org/10.1371/journal.pone.0048392.

[55] W.L. Holland, A.C. Adams, J.T. Brozinick, H.H. Bui, Y. Miyauchi, C.M. Kusminski, S.M. Bauer, M. Wade, E. Singhal, C.C. Cheng, K. Volk, M.-S. Kuo, R. Gordillo, A. Kharitonenkov, P.E. Scherer, An FGF21-Adiponectin-Ceramide Axis Controls Energy Expenditure and Insulin Action in Mice, Cell Metab 17 (2013) 790–797. https://doi.org/10.1016/j.cmet.2013.03.019.

[56] Z. Lin, H. Tian, K.S.L. Lam, S. Lin, R.C.L. Hoo, M. Konishi, N. Itoh, Y. Wang, S.R. Bornstein, A. Xu, X. Li, Adiponectin Mediates the Metabolic Effects of FGF21 on Glucose

Homeostasis and Insulin Sensitivity in Mice, Cell Metab 17 (2013) 779–789. https://doi.org/10.1016/j.cmet.2013.04.005.

[57] C.C. Rusu, S. Racasan, I.M. Kacso, D. Moldovan, A. Potra, D. Tirinescu, C. Budurea, R. Orasan, I.M. Patiu, C.I. Bondor, D. Vladutiu, M.G. Caprioara, The metabolic hormone FGF21 is associated with endothelial dysfunction in hemodialysis patients, Int Urol Nephrol 49 (2017) 517–523. https://doi.org/10.1007/s11255-016-1474-x.

[58] Z. Lin, X. Pan, F. Wu, D. Ye, Y. Zhang, Y. Wang, L. Jin, Q. Lian, Y. Huang, H. Ding, C. Triggle, K. Wang, X. Li, A. Xu, Fibroblast Growth Factor 21 Prevents Atherosclerosis by Suppression of Hepatic Sterol Regulatory Element-Binding Protein-2 and Induction of Adiponectin in Mice, Circulation 131 (2015) 1861–1871. https://doi.org/10.1161/CIRCULATIONAHA.115.015308.

[59] L. Ying, N. Li, Z. He, X. Zeng, Y. Nan, J. Chen, P. Miao, Y. Ying, W. Lin, X. Zhao, L. Lu, M. Chen, W. Cen, T. Guo, X. Li, Z. Huang, Y. Wang, Fibroblast growth factor 21 Ameliorates diabetes-induced endothelial dysfunction in mouse aorta via activation of the CaMKK2/AMPKα signaling pathway, Cell Death Dis 10 (2019) 665. https://doi.org/10.1038/s41419-019-1893-6.

[60] H. Tan, T. Yue, Z. Chen, W. Wu, S. Xu, J. Weng, Targeting FGF21 in cardiovascular and metabolic diseases: from mechanism to medicine, Int J Biol Sci 19 (2023) 66–88. https://doi.org/10.7150/ijbs.73936.

[61] R.-Y. Gao, B.-G. Hsu, D.-A. Wu, J.-S. Hou, M.-C. Chen, Serum Fibroblast Growth Factor 21 Levels Are Positively Associated with Metabolic Syndrome in Patients with Type 2 Diabetes, Int J Endocrinol 2019 (2019) 1–8. https://doi.org/10.1155/2019/5163245.

[62] Y. Tomita, Z. Fu, Z. Wang, B. Cakir, S.S. Cho, W. Britton, Y. Sun, A. Hellström, S. Talukdar, L.E.H. Smith, Long-Acting FGF21 Inhibits Retinal Vascular Leakage in In Vivo and In Vitro Models, Int J Mol Sci 21 (2020) 1188. https://doi.org/10.3390/ijms21041188.

[63] Z. Fu, Y. Gong, R. Liegl, Z. Wang, C.-H. Liu, S.S. Meng, S.B. Burnim, N.J. Saba, T.W. Fredrick, P.C. Morss, A. Hellstrom, S. Talukdar, L.E.H. Smith, FGF21 Administration Suppresses Retinal and Choroidal Neovascularization in Mice, Cell Rep 18 (2017) 1606–1613. https://doi.org/10.1016/j.celrep.2017.01.014.

[64] S. Jin, N. Xia, L. Han, Association between serum fibroblast growth factor 21 level and sight-threatening diabetic retinopathy in Chinese patients with type 2 diabetes, BMJ Open Diabetes Res Care 9 (2021) e002126. https://doi.org/10.1136/bmjdrc-2021-002126.

Figure 1

PRISMA flow diagram of the included studies

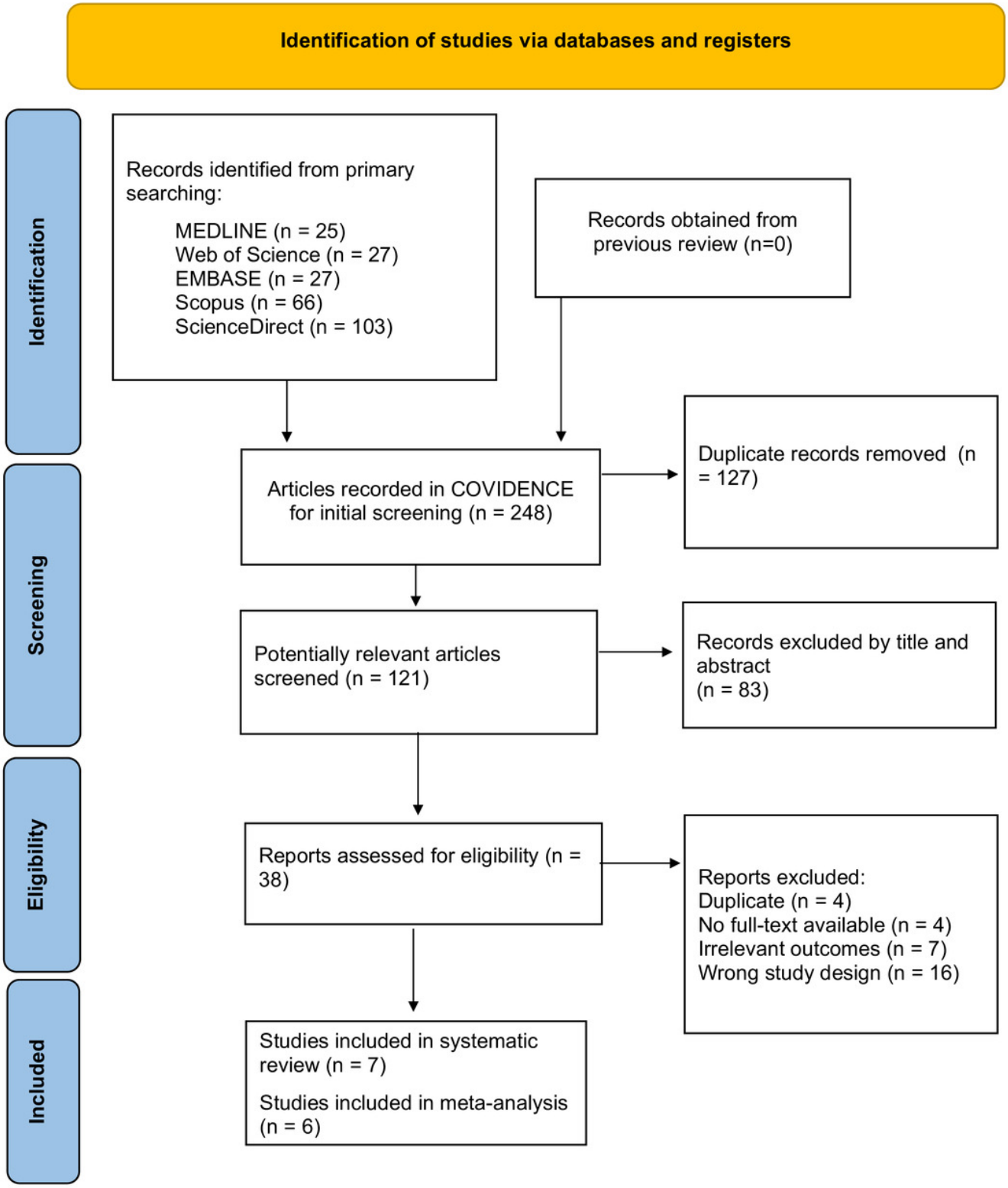
Identification of studies via databases and registers
Identification
Screening
Eligibility
Included
Records identified from primary searching:
MEDLINE (n = 25)
Web of Science (n = 27)
EMBASE (n = 27)
Scopus (n = 66)
ScienceDirect (n = 103)
Records obtained from previous review (n=0)
Articles recorded in COVIDENCE for initial screening (n = 248)
Duplicate records removed (n = 127)
Potentially relevant articles screened (n = 121)
Records excluded by title and abstract (n = 83)
Reports assessed for eligibility (n = 38)
Reports excluded:
Duplicate (n = 4)
No full-text available (n = 4)
Irrelevant outcomes (n = 7)
Wrong study design (n = 16)
Studies included in systematic review (n = 7)
Studies included in meta-analysis (n = 6)

Figure 2

Association between FGF-21 levels with DR incidence

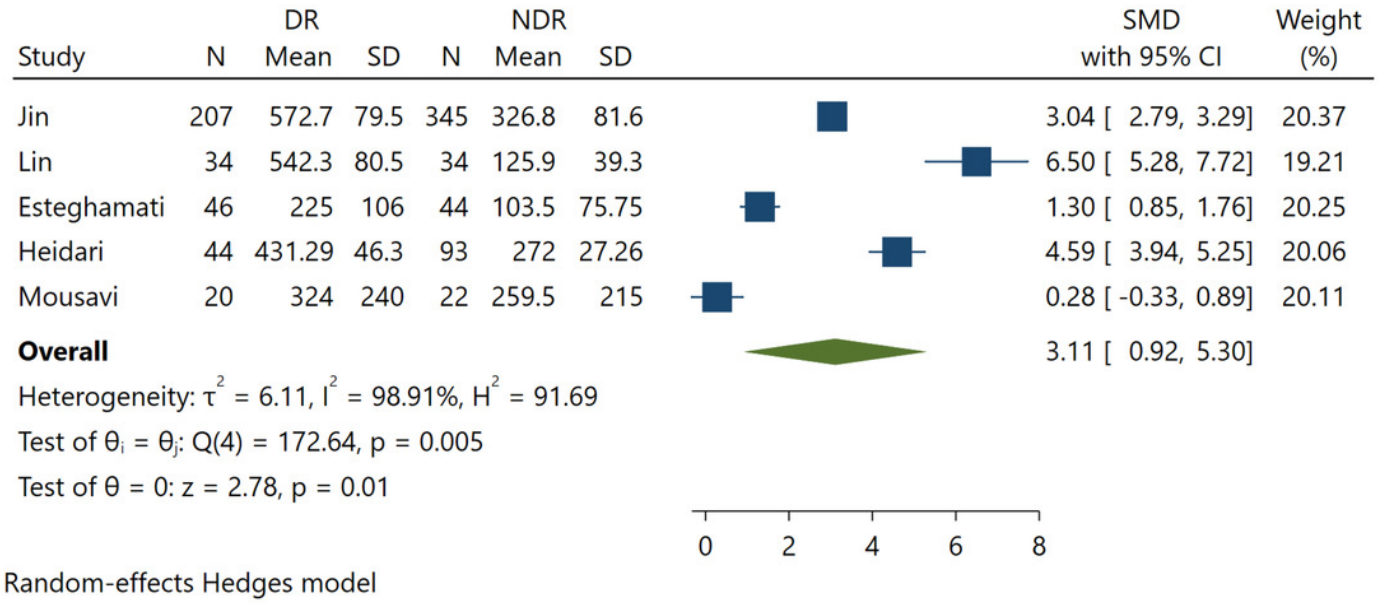

Figure 3

Association between FGF-21 levels with STDR incidence

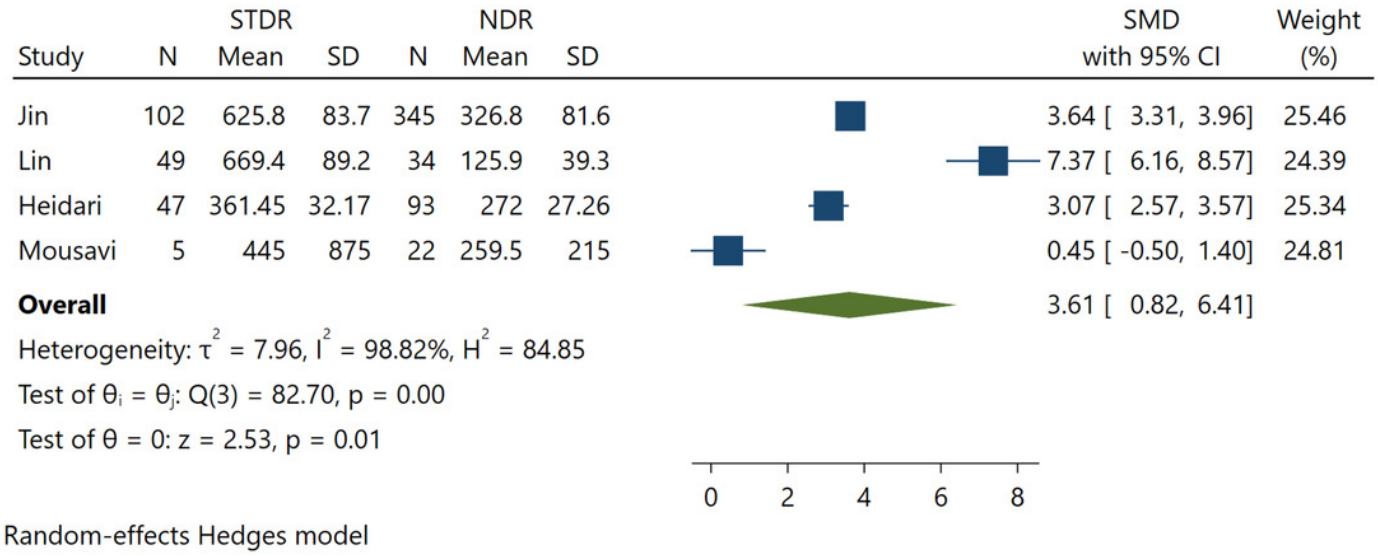

Figure 4

Figure 4. Association between FGF-21 levels with DR severity

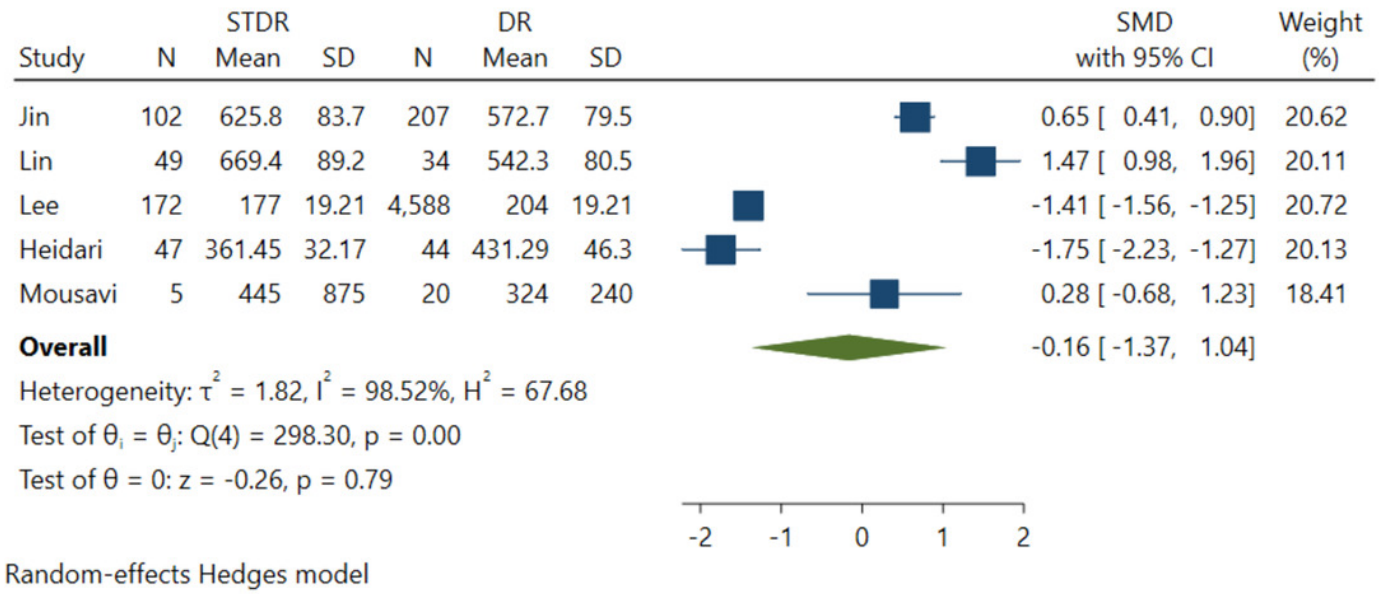

Figure 5

Summary of subgroup analysis

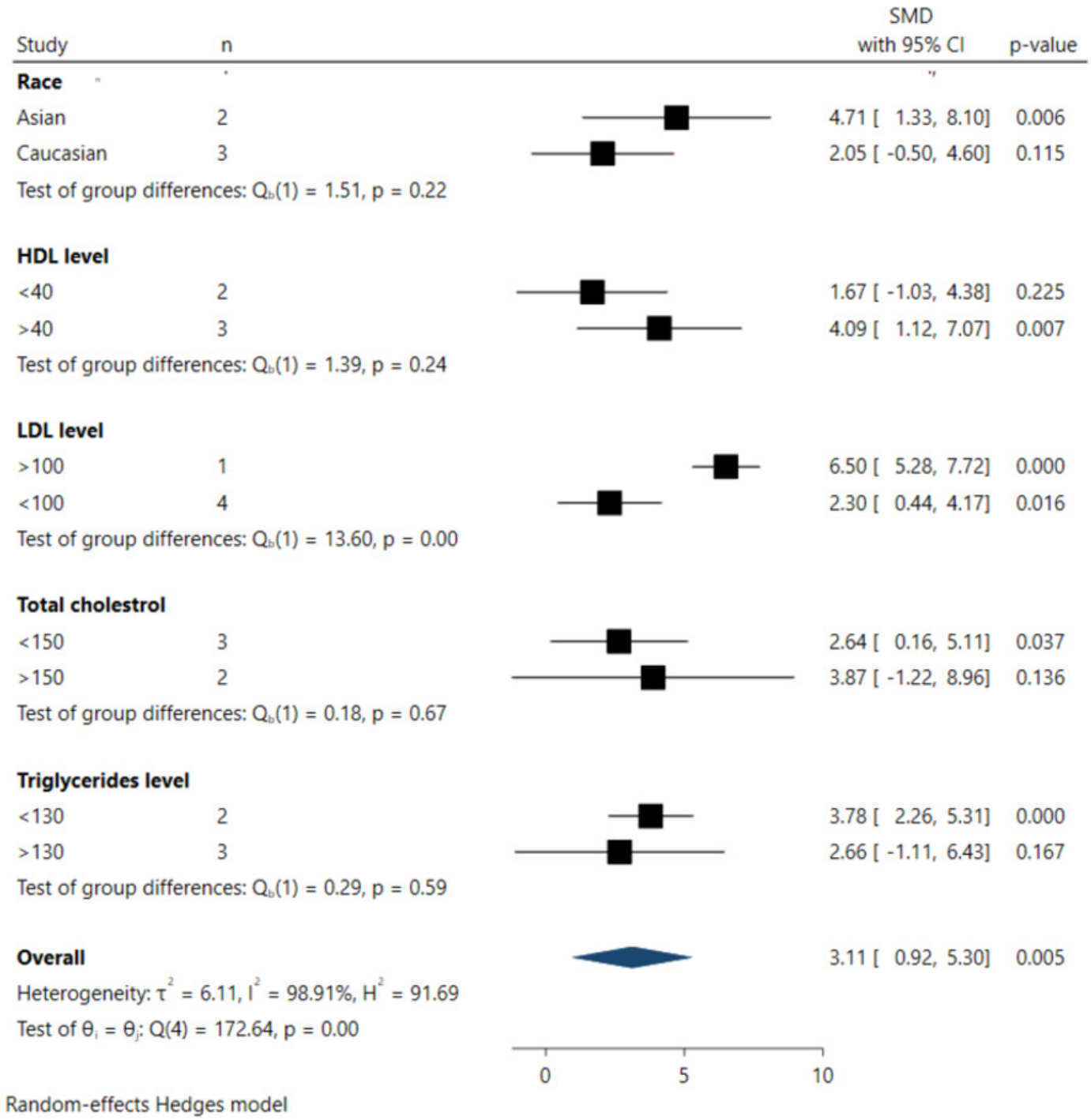

Figure 6

Sensitivity Analysis

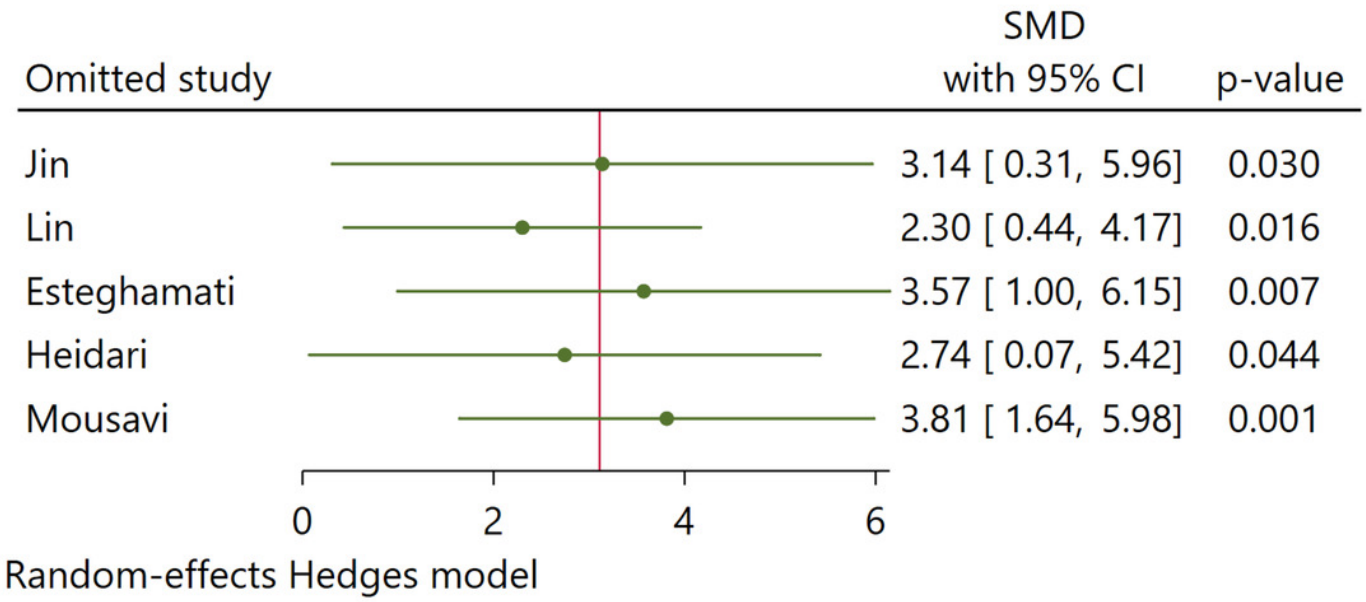

Figure 7

Funnel plot

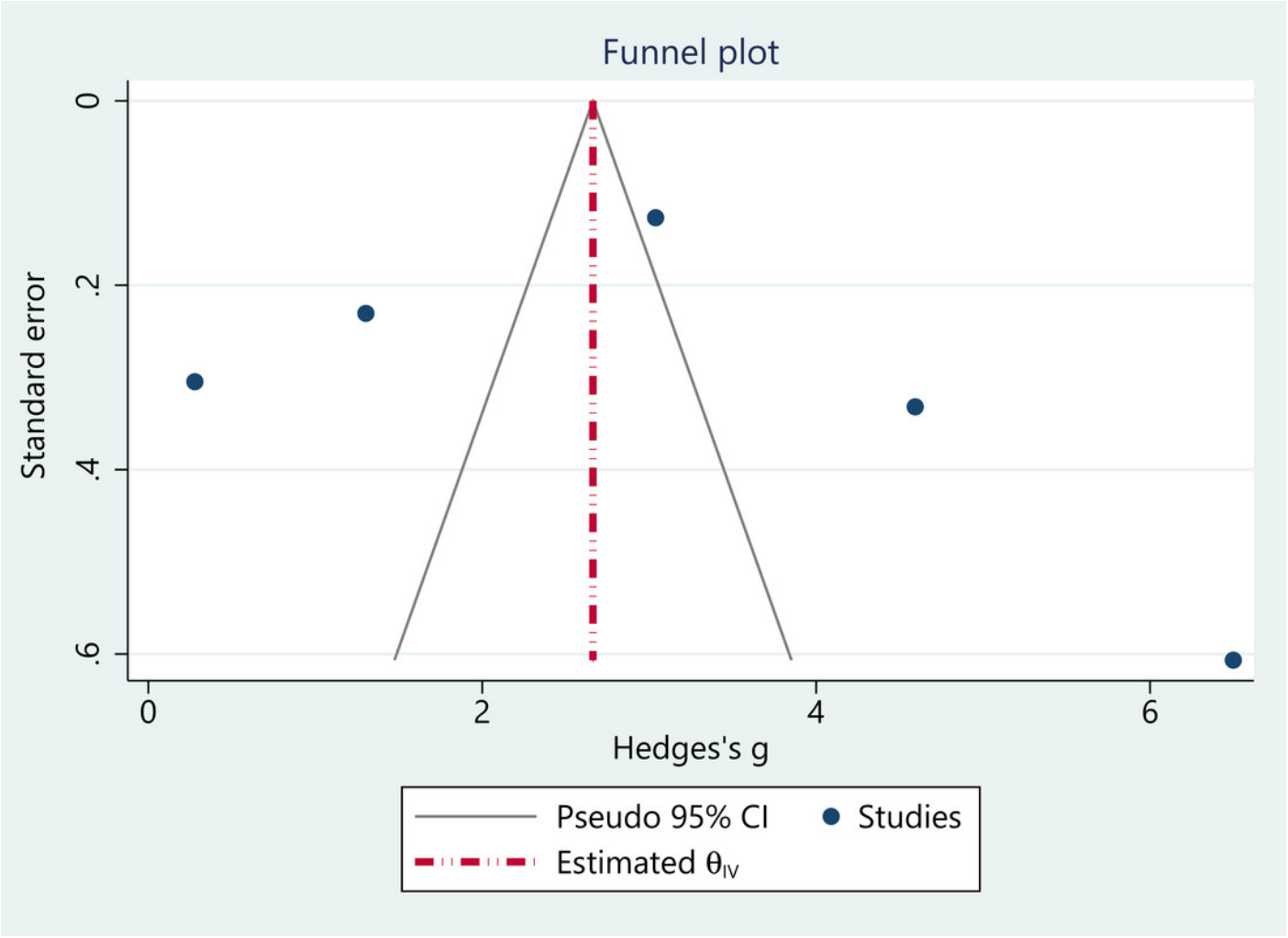

Table 1(on next page)

Characteristics of included study

Author	Year	Country	Study design	Sample size NDR/DR/STDR	FGF-21 cut off value	NOS
Esteghamati [12]	2016	Iran	Cross-sectional	44/46	233.00 (109.00) pg/mL in NPDR and 215.00 (122.00) pg/mL in STDR, (P = 0.361).	8
Heidari [13]	2021	Iran	Cross-sectional	93/44/47	DR prediction with FGF-21 >312 pg/ml, with sensitivity of 97.80% and specificity of 96.77%.	9
Jung [14]	2016	South Korea	Cross-sectional	227	OR for the DR incidence was 0.08 for the FGF21 second tertile when compared with the first tertile (p=0.029). OR of retinopathy in third tertile group was lower than first tertile and higher than second tertile, but statistically insignificant.	7
Jin [15]	2021	China	Cross-sectional	345/207/102	Serum FGF21 level was noted as an independent risk factor for DR and STDR (p<0.01). Serum FGF21 level >478.76 pg/mL suggested the occurrence of DR and that level >554.69 pg/mL indicated STDR (p<0.01).	8
Mousavi [16]	2017	Iran	Cross-sectional	22/25	Serum FGF-21 predicts DR with the cutoff of 196 pg/mL, with a sensitivity of 80 % and specificity of 47.2 %.	7
Lin [8]	2014	China	Cross-sectional	34/34/49	The estimated cut-off value of FGF21 is550 pg/mL, with 86.5% sensitivity and 75% specificity for theexistence of diabetic retinopathy (area under the curve = 0.776,*P* > 0.05).	8
Lee [17]	2023	China	Retrospective	4760	FGF-21 did not significantly	9

			cohort		predict DR incidence (HR 1.10 (0.96-1.26), p = 0.16)	

DR = diabetic retinopathy; NPR = non-proliferative diabetic retinopathy; STDR = sight-threatening diabetic retinopathy

Table 2(on next page)

Summary of meta-regression

Variate	Estimate, 95% CI	p-value
HbA1c	0.03 (-4.65, 4.72)	0.988
Age	0.78 (-0.07, 1.64)	0.075
Sex	0.09 (10.1, 0,28)	0.348
Race	-2.65 (-6.82, 1.50)	0.210
Duration of DM	-0.604 (-7.9, 6.69)	0.871
SBP	0.20 (-1.89, 2.29)	0.851
LDL	-4.19 (-8.51, 0.11)	0.056
HDL	2.42 (-1.90, 6.74)	0.273
TG	-1.16 (-6.15, 3.89)	0.648
TC	1.21 (-3.75, 6.18)	0.633

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