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CLINICAL STUDY

Evaluation of fractalkine (FKN) and secreted frizzled-related protein 4 (SFRP-4) serum levels in patients with prediabetes and type 2 diabetes

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ABSTRACT

OBJECTIVE: The objective of this study is to compare serum levels of FKN and SFRP-4 in patients with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and type 2 diabetes mellitus (T2DM).

METHODS: A total of 152 patients presented to the endocrinology outpatient clinic of our hospital were included in the study. Eighty-two patients with a history of T2DM were assigned to the T2DM group. IGT (n = 34) and NGT (n = 36) groups included the patients who received oral glucose tolerance test outcomes.

RESULTS: Serum FKN levels were significantly higher in the IGT and T2DM groups compared to the NGT group ($p < 0.001$ and $p < 0.001$, respectively). Serum SFRP-4 levels were significantly higher in the T2DM group compared to the IGT and NGT groups ($p = 0.001$ and $p = 0.004$, respectively). A significant correlation was observed between FKN and fasting glucose levels. SFRP-4 was significantly correlated with fasting glucose, HbA1c, and triglyceride levels.

CONCLUSION: To our knowledge, increased FKN levels in patients with IGT were demonstrated for the first time in this study. The results of our study support the opinion that FKN and SFRP-4 may contribute to the pathogenesis of T2DM *(Tab. 1, Fig. 3, Ref. 23)*. Text in PDF *www.elis.sk.*

KEY WORDS: inflammation, insulin secretion, secreted frizzled-related protein 4, fractalkine.

Introduction

Type 2 diabetes mellitus (T2DM) is a heterogeneous disorder characterized by pancreatic β-cell dysfunction and insulin resistance (1). Defect occurrence in β cell functions has been shown in patients newly diagnosed with impaired glucose tolerance (IGT) and T2DM (1). Progressive β cell dysfunction is a condition seen in the natural course of T2DM, but a detailed mechanism has not yet been clarified. Increasing evidence points to chronic low-grade inflammation as the cause of this condition (2–5).

Previous studies have demonstrated abnormally high levels of proinflammatory cytokines such as IL-1β, IL-6, and CRP in T2DM (6). These cytokines may induce a natural immune response in T2DM patients and strengthen inflammatory mechanisms in peripheral tissues as well as pancreatic islet cells. On the other hand, dysfunctional adipocytes in obesity contribute to increased levels of inflammatory cytokines (7). Adipocytokines such as leptin, apelin and TNFα may contribute to the development of β cell damage during insulin resistance, providing a link between adipose tissue inflammation and β cell dysfunction (8, 9).

Two important newly defined cytokines thought to contribute to the link between adipose tissue inflammation and β cell dysfunction, fractalkine (FKN) and secreted frizzled-related protein 4 (SFRP-4), have been the focus of recent studies evaluating their possible contribution to the pathogenesis of T2DM. Despite numerous published studies, further evidence is needed for the clinical use of these cytokines.

The objective of this study is to compare the serum levels of FKN and SFRP-4 in patients with normal glucose tolerance (NGT), IGT, and T2DM.

Materials and methods

A total of 152 patients presented to the endocrinology outpatient clinic of our hospital were included in the study. Patients with acute and/or chronic complications of diabetes, who had undergone cardiovascular intervention within the last six months, with renal or hepatic failure, with malignancies, who were receiving systemic steroid therapy, or who were pregnant or breastfeeding were excluded from the study. Each subject provided written informed consent, and research protocols were approved by the ethical committee of our institution.

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Tab. 1. Demographic features and laboratory results of groups.

Parameter	NGT (n = 36)	IGT (n = 34)	T2DM (n = 82)
Age (years)	51.5±6.8	51.7±5.4	53.6±9.7
N (Male/Female)	10/26	10/24	36/46
Body mass index (kg/m^2)	29.0±2.9	30.2±3.6	31.1±5.4
Systolic blood pressure (mmHg)	118±8	120±12	128±13[ac]
Diastolic blood pressure (mmHg)	67±7	70±10	81±11[ac]
Fracktalkine (ng/mL)	0.24 (0.02–0.94)	0.61[b] (0.35–0.79)	0.53[b] (0.18–1.05)
SFRP–4 (ng/mL)	0.170 (0.12–0.45)	0.183 (0.13–0.70)	0.282[ad] (0.11–2.51)
Fasting glucose (mg/dL)	90 (8–103)	106 (92–125)[b]	140 (88–388)[bd]
Fasting insulin (mIU/mL)	7.54 (2.25–13.56)	10.91[a] (2.78–32.30)	9.99[a] (1.18–47.50)
HbA1c (%)	5.35 (40–321)	5.7 (5–6.2)[a]	7.2 (5.2–14.3)[bd]
Total cholesterol (mg/dL)	193±43	203±37	190±44
LDL-C (mg/dL)	119±35	129±33	114±38
HDL-C (mg/dL)	47±11	45±8	45±11
Triglycerides (mg/dL)	98 (40–321)	136 (36–363)	160 (40–619)[b]

[a]$p < 0.01$; [b]$p < 0.001$ compared with NGT; [c]$p < 0.01$; [d]$p < 0.001$ compared with BGT. NGT – normal glucose tolerance, BGT – impaired glucose tolerance, T2DM – type 2 diabetes mellitus, SFRP-4 – secreted frizzled-related protein 4

Eighty-two patients with a history of T2DM were assigned to the T2DM group. IGT (n = 34) and NGT (n = 36) groups included the patients who received an oral glucose tolerance test due to risk factors within the last three months, and the patients were sorted into groups according to test outcomes. Criteria recommended by the World Health Organization in 1999 were used for the diagnosis of NGT, IGT, and T2DM (10).

Age, gender, height, weight, body mass index (BMI), additional diseases, and diabetes duration of the included patients were recorded.

Venous blood samples were collected from the patients between 8:00 and 9:00 in the morning following one-night fasting. The samples were centrifuged at 4 °C, and the serum samples were kept at −80°C until biochemical analysis was done. Serum glucose, insulin, and HbA1c were measured with hexokinase (Abbott ARCHITECT, Abbott Laboratories, Chicago, IL, USA), immunochemiluminescence (Roche E170, Roche Diagnostics, Indianapolis, IN, USA), and ion-exchange chromatography (TOSOH G7, Tosoh Bioscience, San Francisco, CA, USA), respectively. The triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) concentrations were assayed using enzymatic methods.

Serum FKN and SFRP-4 measurements were performed using specific enzyme-linked immunosorbent assay (ELISA) immunoassay kits (Catalog No: DCX310, R&D Systems, Minneapolis, MN, USA, and Catalog No: MBS764667, MyBioSource, San Diego, CA, USA, respectively). The manufacturer's protocols for the use of the kits were followed.

Statistical analysis of the data was performed utilizing SPSS version 20.0 software. A one-way ANOVA test was used for calculating the mean for each of the three groups in parametric variables, the Kruskal–Wallis test was used to obtain the median values for each of the three groups in non-parametric variables, and a Bonferroni corrected Mann–Whitney U test was used for a post hoc test comparison between the groups. Correlation analysis between FKN, SFRP-4, and other parameters was carried out with Spearman's correlation test. Parametric data were expressed as mean ± standard deviation, and non-parametric data were accordingly shown as median (minimum–maximum) values. Because three groups were compared, $p < 0.017$ were considered statistically significant ($p < 0.05/3$).

Results

Clinical characteristics and laboratory values of the groups are given in Table 1. No significant differences were observed between the groups in terms of age, gender, BMI, TC, LDL-C, and HDL-C values. TG levels were significantly higher in the T2DM group than in the NGT group.

HbA1c and fasting glucose levels were significantly higher in T2DM and IGT patients as compared to the NGT group. HbA1c and fasting glucose levels were significantly higher in T2DM patients than in IGT patients. Fasting insulin levels were significantly higher in the IGT and T2DM groups as compared to the NGT group. No significant difference was observed between the IGT and T2DM groups in terms of fasting insulin levels ($p = 0.846$).

Serum FKN levels were significantly higher in the IGT and T2DM groups than in the NGT group ($p < 0.001$ and $p < 0.001$, respectively). No significant difference was observed between the IGT and T2DM groups in terms of serum FKN levels ($p = 0.656$).

Serum SFRP-4 levels were significantly higher in the T2DM group compared to the IGT and NGT groups ($p = 0.001$ and $p =$

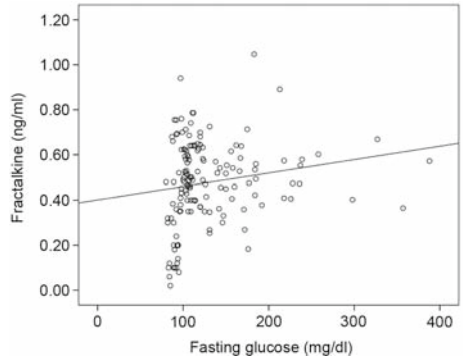

Fig. 1. Correlation between fractalkine (FKN) and fasting glucose.

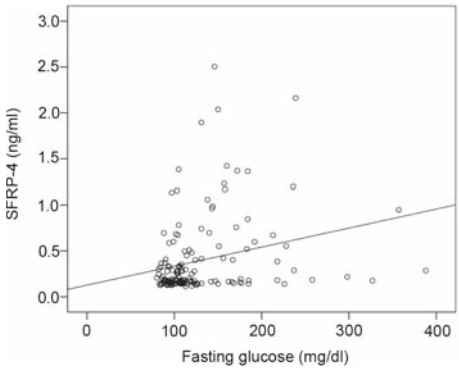

Fig. 2. Correlation between secreted frizzled-related protein 4 (SFRP--4) and fasting glucose.

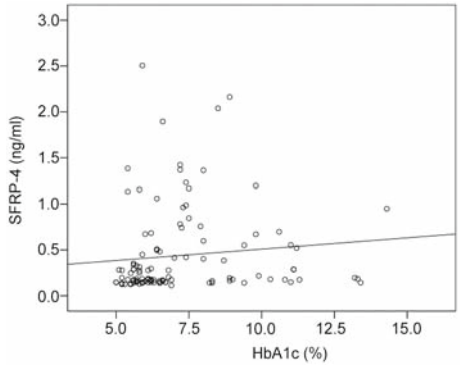

Fig. 3. Correlation between secreted frizzled-related protein 4 (SFRP--4) and HbA1c.

0.004, respectively). SFRP-4 levels were not significantly different in the IGT and NGT groups (p = 0.630).

A significant correlation was found between FKN and fasting glucose levels (r = 0.299, p = 0.004) (Fig. 1). SFRP-4 was significantly correlated with fasting glucose (r = 0.274, p = 0.001), HbA1c (r = 0.291, p = 0.002), and TG (r = 0.199, p = 0.015) levels (Figs 2 and 3).

Discussion

T2DM is closely associated with obesity and adipose tissue inflammation. Monocyte accumulation in adipose tissue plays a major role in the development process of T2DM, which initiates production of local cytokines, causing the development of systemic inflammation and insulin resistance (11). The dual effects of FKN on leukocyte chemotaxis and on adhesion mechanisms may be a pathogenic factor causing adipocyte dysfunction and T2DM (12). However, data on this subject are scarce and include some differences.

Shah et al clearly confirmed expression and secretion of FKN in human adipocyte cells and therefore considered FKN to be an adipocytokine (13). In that study, Shah et al found a significant increase in both adipose tissue and plasma levels of FKN with moderate levels of endotoxemia. It was found in the same study that monocyte adhesion to FKN-blocking antibodies and adipocytes had decreased by about 50%. In the case control arm of that study, plasma FKN levels were found to be significantly lower in patients without diabetes than in those with T2DM. In another study, Mehta et al showed that FKN mRNA levels were increased 15-fold after administration of lipopolysaccharide, which was found to be a greater increase than the other cytokines (IL-6, TNF-α, and MCP-1) (14).

In contrast to these studies, Lee et al demonstrated that the FKN/CX3CR1 signaling pathway plays a regulatory role in pancreatic β cell functions and improves insulin secretion and glucose intake in mice and human islet cells in vitro (15). In that study, expression of FKN in the islet cells decreased with a high-fat diet, obesity, and aging, and the authors summarized that low levels of FKN/CX3CR1 might contribute to β cell dysfunction in T2DM. Furthermore, that study observed increased insulin secretion and improved glucose tolerance with in vivo FKN administration. Given those results, the authors stated that the use of FKN-based therapeutic agents could be considered.

A plausible explanation for the contradictory data obtained from these studies is that expression of CX3CR1 in β cells of T2DM patients may be decreased, and the increase in FKN could be due to the development of a response to compensate for that situation. In support of this hypothesis, a pattern of high serum levels of FKN and decreased levels of receptors has been demonstrated in chronic liver disease (16).

In our study, significantly higher FKN levels were found in the IGT and T2DM patients as compared to the NGT group. No significant difference was observed between IGT and T2DM patients in terms of FKN levels. To our knowledge, this study was the first to demonstrate increased FKN levels in IGT patients. Considering that IGT indicates a prediabetic period that progresses with increased insulin levels, it could be hypothesized that the increased FKN levels in the IGT patients in our study may reflect a compensatory increase corresponding to the decreased tissue levels of CX3CR1 before the disease.

The Wnt signaling pathways are involved in many critical mechanisms, including cell proliferation and migration (11). Some components of the Wnt signaling pathways are associated with lipid and glucose metabolism (11). Therefore, the Wnt signaling pathways may play a crucial role in the pathogenesis of metabolic diseases.

SFRP-4 is a member of the protein family acting as a modulator of Wnt signaling pathways and a recently discovered inflammatory cytokine that has a G-coupled receptor (17). The relationship of SFRP-4 with β cell dysfunction in T2DM is closely associated with the expression of SFRP-4 in the pancreatic islet cells (11). It has been demonstrated in a series of studies that SFRP-4 is a Wnt antagonist and inhibits Wnt signaling systems in cancer cells and adipocytes (18). In contrast, however, it is believed that the activation of Wnt signaling pathways may be one of the major pathogenic factors in pancreatic cells related to the pathogenesis of T2DM (19). Increased activation of Wnt signaling pathways may play an adaptive role, as seen in the example of increased β cell proliferation in the early periods of T2DM. On the other hand, chronic activation of Wnt signaling pathways stimulates cell apoptosis, decreasing insulin secretion (11). Therefore, increased SFRP-4

expression in β cells of diabetic patients could be considered to be a result of chronically deteriorated Wnt signaling pathways.

Despite these interesting hypotheses, the role of SFRP-4 in obesity and T2DM pathogenesis is not yet fully understood. Ehrlund et al showed that mRNA expression of SFRP-4 and secretion of this protein from visceral adipose tissue is increased in obesity and that this increase is correlated with decreased insulin secretion (20). Taneera et al demonstrated a negative correlation between SFRP-4 expression and insulin secretion and a positive correlation between glycosylated hemoglobin (HbA1c) levels in human pancreatic islet cells (17). Mahdi et al reported that SFRP-4 expression is associated with inflammation and defective insulin secretion, and therefore, SFRP-4 could be considered a biomarker for pancreatic islet dysfunction in T2DM (21). Also, in recent clinical studies SFRP-4 has been shown to significantly increase in T2DM and BGT patients compared to healthy subjects (22, 23).

In our study, serum levels of SFRP-4 were found to be significantly higher in T2DM patients compared to the IGT and NGT groups, which is consistent with the studies in the literature. On the other hand, no significant difference was found between the NGT and IGT groups. However, this result may be due to the small number of our patients in IGT group.

This study has several limitations, such as the groups having a relatively small number of patients, the inability to study important proinflammatory cytokines such as IL-1β, and the lack of long-term follow-up for outcomes and serial measurements.

Conclusion

The results of this study support the hypothesis that deteriorated FKN/CX3CR1 and Wnt signaling pathways that are disrupted by SFRP-4 may be among the causes of the chronic low-grade inflammation that plays an important role in the pathogenesis of T2DM. We believe that the place of FKN and SFRP-4 cytokines in the pathogenesis of T2DM should be determined in order to determine if they are suitable drug targets in treatments aimed at improving insulin secretion and to consider their use as an early biomarker for β cell dysfunction.

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