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CLINICAL STUDY

Prorenin and secreted frizzled-related protein 4 levels in women with gestational diabetes mellitus

Baldane S[1], Ipekci SH[1], Kebapcilar AG[2], Abusoglu A[3], Beyhekim H[2], Ilhan TT[2], Unlu A[3], Kebapcilar A[1]

Division of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, Selcuk University, Konya, Turkey. **baldane42@hotmail.com**

ABSTRACT

OBJECTIVE: This study was designed to investigate prorenin and secreted frizzled-related protein 4 (SFRP4) levels in pregnancies with or without gestational diabetes mellitus (GDM).

METHODS: A total of 76 pregnant women were included in the study. Thirty-five of the pregnant women were included in GDM group according to the results of oral glucose tolerance tests (OGTT) and 41 of them were included in the control group.

RESULTS: In the group with GDM, SFRP4 value was found to be significantly higher than that of the control group (5.59 ± 3.32 ng/mL vs 4.05 ± 2.15 ng/mL; p = 0.017). Women with GDM had significantly higher serum prorenin levels compared with control group [737 (427-1339) pg/mL vs. 535 (376-725) pg/mL; p = 0.009]. There was a significant positive association between prorenin and SFRP4 levels in GDM (r = 0.91; p < 0.001) and control groups (r = 0.42; p = 0.002) and whole pregnancies (r = 0.75; p = 0.002).

CONCLUSION: We have shown that prorenin and SFRP4 were significantly elevated in GDM patients when compared to healthy control group. Furthermore, we found that there was a positive correlation between prorenin and SFRP4 *(Tab. 1, Fig. 2, Ref. 38)*. Text in PDF *www.elis.sk.*

KEY WORDS: gestational diabetes mellitus, prorenin, SFRP 4, subclinical inflammation.

Introduction

Women with gestational diabetes mellitus (GDM) are characterized by increased serum angiotensin II (Ang II) levels. Increased prorenin levels promote pathogenesis of diabetes through Ang II poduction. Recent animal and human studies suggest that Ang II contents contributes to the development of insulin resistance in skeletal muscle tissues and induces subclinical inflammation and new-onset diabetes mellitus (1–3). In addition, the level of (pro) renin receptor was reported to be high in pregnant women with GDM compared to healthy pregnant women in a s number of studies (4–6). Having higher levels of prorenin in diabetic patients is reported by previous studies (7–10).

On the other hand, secreted frizzled-related protein 4 (SFRP-4) reduces insulin secretion and increased proinflammatory factors (11–14). Increased SFRP4 in diabetic subjects can induce IL-1β concentrations (15). SFRP-4 is also expressed in the placenta (16–18). Increasing evidence suggests that the increase in proinflammatory factors plays an important factor in the development of GDM (19–21). Therefore, an elevation of SFRP-4 may be a good marker of beta cell dysfunction and it can play an important risk factor in the pathogenesis of GDM.

To our knowledge SFRP-4 levels have never been investigated in patients with GDM. The aim of this study was to compare prorenin and SFRP-4 levels in pregnant women with GDM and healthy pregnant women and to evaluate the association between the levels of prorenin and SFRP-4.

Materials and methods

Subjects

The present study was a cross-sectional, case-control study. All patients participating in the study signed an informed consent form. The study was approved by our institutional ethics committee.

All recruited pregnant women were selected randomly from women admitted to our gynecology and endocrinology outpatient clinic between January 2015 and June 2016. Women with a twin pregnancy, prior cardiovascular disease, pregestational diabetes, those taking any medication or suffering from gestational or preexisting hypertensive disorders, renal or thyroid diseases, smokers, and those with acute or chronic infections were excluded.

Oral glucose tolerance test (OGTT) was performed in all pregnant women included in the study between weeks 24 and 28 of pregnancy using 75 g of glucose. Pregnant women were diagnosed to have GDM when one or more of the test results were abnormal

[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, Selcuk University, Konya, Turkey, [2]Department of Obstetrics and Gynaecology, Faculty of Medicine, Selcuk University, Konya, Turkey, and [3]Department of Biochemistry, Faculty of Medicine, Selcuk University, Konya, Turkey

Address for correspondence: S. Baldane, Dr, Division of Endocrinology and Metabolism, Department of Internal Medicine, Faculty of Medicine, Selcuk University, Konya, 42075, Turkey.
Phone: +903322244685, Fax: +903322412184

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according to the recommendations of American Diabetes Association (ADA; fasting ≥ 92 mg/dL, ≥ 180 mg/dL after 1 h, and ≥ 153 mg/dL after 2 h; 22). Age, gestational age and body mass index of all pregnant women were recorded.

Blood sampling and assay

The blood samples were allowed to clot at room temperature and then centrifuged at room temperature for 10 min at 2,000 g. All tubes were stored at –80 °C until analysis.

Serum glucose, total cholesterol, triglycerides and HDL-cholesterol levels were measured using Abbott Architect C-8000. LDL-cholesterol was derived by the Friedewald equation method. Glucose levels were measured using a hexokinase method and Abbott Architect C-8000 device.

SFRP-4 and prorenin assay

Serum SFRP-4 levels were measured using specific enzyme-linked immunosorbent assay (ELISA) immunoassay kit (Mybiosource, San Diego, CA, USA). Serum prorenin concentrations were measured using an ELISA kit (LINCO Research Inc., St. Charles, MO, USA).

Statistical analysis

The Kolmogorov–Smirnov normality test was used to determine the distribution pattern of the variables. Results were presented as mean ± standard deviation. A comparison between groups of continuous variables was performed using a Student's t test or a Mann-Whitney U test. Differences between categorical variables were analyzed using a Fisher's exact test. Relationships between variables were analyzed using Pearson's or Spearman's rank correlation coefficients. Binary logistical regression analysis was used to identify the factors associated with the presence of GDM. The statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS), version 20.0 (SPSS Inc., Chicago, IL, USA). A p-value of < 0.05 was considered statistically significant.

Results

A total of 76 pregnant women were included in the study. Thirty-five of the pregnant women were included in the GDM group according to the results of OGTT, and 41 of them were included in the control group.

There were no significant differences in age, gestational age, BMI and lipid profile levels between the two groups (Tab. 1). However fasting blood glucose (FBG), prorenin and SFRP-4 were significantly higher in individuals with GDM (Tab. 1). The mean FBG level of GDM group was 91.54 ± 15.11 mg/dL and mean FBG level of control group was 77.94 ± 5.88 mg/dL ($p < 0.001$).

In the group with GDM, SFRP4 value was found to be significantly higher than that of the control group (5.59 ± 3.32 ng/mL vs 4.05 ± 2.15 ng/mL; $p = 0.017$). Women with GDM had significantly higher serum prorenin levels compared with the control group [737 (427-1339) pg/mL vs. 535 (376-725) pg/mL; $p = 0.009$].

Tab. 1. Clinical and laboratory characteristics of GDM and control groups.

Parameter	GDM (n=35)	Control (n=41)	p
Age (years)	30.11±4.26	29.14±4.83	0.3
Gestational age (weeks)	26.04±4.66	25.23±3.75	0.652
BMI (kg/m^2)	29.07±3.26	28.33±4.01	0.1
FBG (mg/dL)	91.54±15.11	77.94±5.88	<0.001
Total cholesterol (mg/dL)	240.10±48.35	230.72±41.30	0.3
LDL cholesterol (mg/dL)	135.81±46.13	128.27±31.71	0.4
HDL cholesterol (mg/dL)	61.04±13.25	61.31±10.51	0.9
Triglycerides (mg/dL)	229.50±76.33	194.85±69.21	0.05
Prorenin (pg/mL)	737 (427–1339)	535 (376–725)	0.009
SFRP- 4 (ng/mL)	5.59±3.32	4.05±2.15	0.017

GDM – gestational diabetes mellitus, BMI – body mass index, FBG – fasting blood glucose, LDL – low-density lipoprotein, HDL – high-density lipoprotein

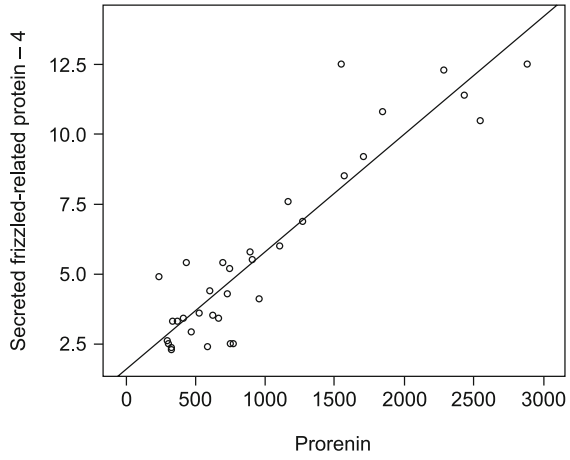

Fig. 1. Correlation between prorenin and SFRP4 levels in GDM.

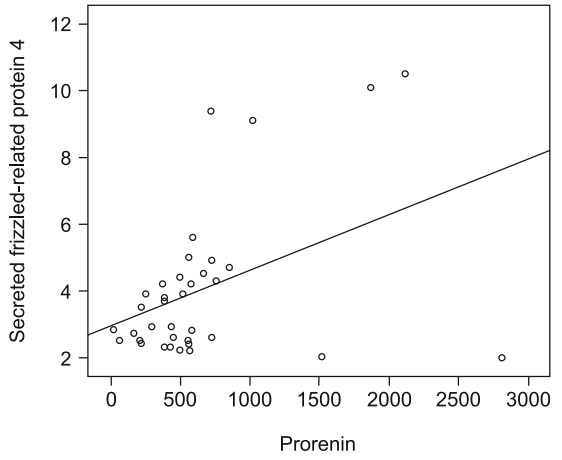

Fig. 2. Correlation between between prorenin and SFRP4 levels in the control group.

There was a significant positive association between prorenin and SFRP4 levels in GDM ($r = 0.91$; $p < 0.001$) (Fig. 1) and control groups ($r = 0.42$; $p = 0.002$) (Fig. 2) and whole whole pregnancies ($r = 0.75$; $p = 0.002$). No other feature was significantly associated with prorenin and SFRP-4.

A significant independent association was found between plasma SFRP-4 and GDM in the binary logistic regression analysis after adjusting for age, BMI, and lipid profile [odds ratio (OR): 5.872; 95% confidence interval (CI); p = 0.015]. A significant independent association was found between plasma prorenin and GDM in the binary logistic regression analysis after adjusting for age, BMI, and lipid profile (OR: 4.182; 95% CI; p = 0.041).

Discussion

In the present study, prorenin and SFRP-4 concentrations were higher in women with GDM and a positive association between the prorenin and SFRP-4 in GDM and non-GDM women was exhibited.

The relationship between prorenin receptor (4–6), prorenin levels (7–10), and diabetes mellitus has been demonstrated in previous studies. Similarly, we found a higher concentration of prorenin levels in GDM group when compared to non-GDM group. Increased plasma prorenin activity has also been described in subjects who had diabetes with complications (8, 23, 24). The association of diabetic organ injury and increase in plasma prorenin has not been clearly established yet. Prorenin was demonstrated to initiate an inflammation via extracellular signal-related protein kinases 1 and 2 (ERK 1/2) and increased mitogen-activated protein kinase activity (25–26). Thus MAP kinases are modulating IRS-1 and GLUT4 protein levels. In humans, decreased GLUT4 and IRS-1 protein abundance can induce the development of type 2 diabetes mellitus (27–28).

Nagai et al. investigated that the increase in (pro)renin receptor in skeletal muscle promotes the development of insulin resistance in animal model (29). Rafiq et al. reported that (pro)renin receptor could induce a decrease in insulin secretion by causing beta-cell dysfunction in the pancreas (30). Recently, one of the studies found that an increase in prorenin levels during the first trimester may induce the development of GDM (6). Bokuda et al. reported that prorenin may contribute to the development of β-cell dysfunction through RAS, ERK 1/2 and MAP kinases activations (4). Wnt-β-catenin pathway is involved in diabetes mellitus (31). Recently, (pro)renin receptor was determined to be an accessory subunit for vacuolar (V-ATPase), which contributes to the activation of the canonical Wnt-β-catenin signaling pathway (32). WNT/β-catenin is activated in epithelial cells, along with significant increases in IL-1β levels (33). Our study results suggest that a high prorenin levels may be a potential risk factor for GDM.

On the other hand, SFRP-4 levels were higher in women with GDM. Our study revealed a relationship between SFRP-4 and prorenin. Mahdi et al. observed high overexpression of SFRP-4 in islets of diabetic subjects and its relationship with inflammatory markers, particularly IL-1β stimulating its production (15). Mahdi et al (15) and Bergmann K et al (14) reported that high expression of SFRP-4 was associated with inflammation and decreased insulin secretion, therefore it seems to be serving as a biomarker of the pancreatic islet dysfunction in T2DM. Increased SFRP-4 concentrations suggest that SFRP-4 could be used potentially as indicators of risk factor for developing glucose intolerance in GDM women. SFRP-4 has been variably reported to activate or inhibit Wnt signaling in different tissues (33-35).

SFRP-4 levels were positively correlated with prorenin levels in GDM and non-GDM groups. It is not surprising that prorenin can induce the same proinflamatory factors such as WNT/b-catenin signaling pathway, RAS, ERK 1/2, IL-1β, and MAP kinases activations, as well as SFRP-4. The proinflammatory cytokines IL-1b function as novel WNT/b-catenin targets. IL-1 β indirectly activates canonical Wnt signaling by up-regulation of Wnt ligands. Overexpression of WNT/b-catenin led to acute inflammation and IL-1 β up-regulates inflammation through activation of the NF kappa B pathway (36–38).

Some limitations should be considered when our results are interpreted. WNT/b-catenin signaling pathway, IL-1β activations and other inflammation markers could be performed to correlate these markers with the risk of diabetes mellitus in women with GDM.

Conclusion

We demonstrated an increase in prorenin and SFPR 4 in patients with GDM. Early intervention in GDM is important because it can improve the maternal glucose intolerance while prorenin and SFRP-4 may be important GDM predictors.

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