10 ISSN:2149-455X
Makale / Article

Is There A Relationship Between Selenoprotein P1 (Sepp1) Gene Polymorphism And Gestational Diabetes Mellitus In Turkish Women Gestasyonal Diyabet Mellituslu Türk Kadinlarini Ve Selenoprotein P1 (Sepp1) Gen Polimorfizmi Arasindaki İlişki Var Midir?

Ayşe Yücel[1], F. Behice Cinemre[2], Hakan Cinemre[3], M. Aytaç Yüksel[4], Abdullah Tüten[4], Nevin Yılmaz[4], Birsen Aydemir[1]*

[1] Sakarya Üniversitesi, Tıp Fakültesi, Biyofizik Anabilim Dalı, Sakarya

[2] Sakarya Üniversitesi, Tıp Fakültesi, Tıbbi Biyokimya Anabilim Dalı, Sakarya

[3] Sakarya Üniversitesi, Tıp Fakültesi, İç Hastalıkları Anabilim Dalı, Sakarya

Abstract

Background: Diabetes Mellitus (DM) is a complex disease caused by a combination of genetic and environmental factors. Selenoprotein P (SeP) appears to play a key role in the etiopathogenesis of DM. Recently, it has been demonstrated that SeP played an important role in glucose metabolism and the regulation of insulin sensitivity as a new hepatokine. The purpose of this study was to determine whether common variations in selenoprotein P1 (SEPP1) alter the risk of Gestational Diabetes Mellitus (GDM).

Methods: 72 pregnant women with GDM and 64 healthy pregnant women from the same geographic region were included in the study. Allele-specific Polymerase Chain Reaction (ASPCR) analysis was used to identify polymorphisms of the SEPP1 gene (rs3877899).

Results: We found that fasting glucose, insulin, HOMA-IR, HbA1c, total cholesterol levels and weight of fetus were higher in gestational diabetic pregnants compared group to healthy pregnant women group. The frequencies of the AA, GA and GG genotypes were found as 28 %, 43 % and 29 % in pregnant women with GDM and 24 %, 50 % and 26 % in healthy pregnant women, respectively. Our results indicated that the distribution of the SePP1 genotypes and alleles did not differ significantly among subjects with or without GDM ($p>0.05$).

Conclusion: Although SeP plays a key role in glucose metabolism and the regulation of insulin sensitivity, the SEPP1 polymorphism did not changed occurrence of GDM in our population. Different mechanisms may be involved in etiopathogenesis of GDM. However, it should be clarified with further studies in larger populations. SEPP1 (rs3877899) polymorphism has no role in development of gestational diabetes in Turkish women.

Keywords: Gestational diabetes mellitus; Selenoprotein P gene; Polymorphism

Özet

Amaç: Diabetes Mellitus (DM) genetik ve çevresel faktörlere bağlı kopleks bir hastalıktır. Selenoprotein P (SeP) DM'nin etyopatogenezinde anahtar rol oynamaktadır. Son zamanlarda, SeP'nin glikoz metabolizmasının ve yeni bir hepatokin olarak insülin duyarlılığının düzenlenmesinde önemli bir rol oynadığı gösterilmiştir. Bu çalışmanın amacı, selenoprotein P1'in (SEPP1) yaygın varyasyonlarının Gestasyonel Diabetes Mellitus (GDM) riskini değiştirip değiştirmediğini saptamaktır.

Materyal ve Metod: Aynı coğrafik bölgeden 72 GDM'li ve 64 sağlıklı gebe çalışmaya dahil edildi. SEPP1 geninin polimorfizmini (rs3877899) belirlemek için allel-özgü Polimeraz Zincir Reaksiyonu (ASPCR) analizi kullanıldı.

Bulgular: Gestasyonel diyabetik gebe grubunda açlık glikozu, insülini, HOMA-IR, HbA1c, total kolesterol düzeyleri ve fetal ağırlık sağlıklı gebe kadın gruba göre daha yüksek bulundu. AA, GA ve GG genotiplerinin frekansları, GDM'li gebelerde sırasıyla % 28, % 43 ve% 29, sağlıklı gebelerde % 24, % 50 ve % 26 olarak bulundu. Bulgularımız, SePP1 genotiplerinin ve allelerinin dağılımının, GDM'si olan ve olmayan kişiler arasında anlamlı farklılık göstermediğini ortaya koymuştur ($p> 0.05$).

Sonuç: SeP, glukoz metabolizması ve insülin duyarlılığının düzenlenmesinde anahtar rol oynamasına rağmen, SEPP1 polimorfizmi popülasyonumuzda GDM oluşumunu değiştirmemiştir. GDM etyopatogenezinde farklı mekanizmalar yer alabilir. Bununla birlikte, daha geniş popülasyonlarda yapılan daha ileri çalışmalarla gereksinim vardır. SEPP1 (rs3877899) polimorfizminin Türk kadınlarında gestasyonel diyabet gelişiminde rolü bulunmamaktadır.

Anahtar Kelimeler: Gestasyonel diyabetes mellitus, selenoprotein P gen, polimorfizm

Corr. Author: **Doç Dr. Birsen Aydemir**
Sakarya Üniversitesi, Korucuk Kampüsü, Tıp Fakültesi, Biyofizik Anabilim Dalı, Adapazarı 54290, Sakarya, Türkiye
T: +90 264 295 31 19 E-mail: **baydemir@sakarya.edu.tr**

Introduction

Gestational diabetes (GDM) is defined as glucose intolerance that is first diagnosed during pregnancy.[1] GDM, which affects 16–20 % of all pregnancies has serious adverse perinatal outcomes and increases long-term risk for the development of obesity, type 2 diabetes and cardiovascular disease in both the mother and the offspring.[2] The underlying mechanisms of diabetic complications are usually related with complications caused by oxidative stress, inflammation and a defective genetic background.[3] It is known that increased production of reactive oxygen species may play a role in all diabetic diseases. On the basis of this hypothesis, in various studies, it was investigated whether the genetic polymorphisms in the antioxidant enzyme affect the sensitivity of diabetes. Several genes were shown to be associated with GDM. In these studies, our aim was to investigate relationship of the genes encoding selenocysteine selenoproteins with occurrence of GDM. One of these selenoproteins is Selenoprotein P (SeP). SeP functions as both Se transporter and antioxidant. Additionally, it has been demonstrated that SeP played an important role in glucose metabolism and the regulation of insulin sensitivity as a new hepatokine. Selenoprotein P (SeP) appears to play a key role in the etiopathogenesis of DM. The human SePP gene (SEPP1) contains several functional polymorphisms, including rs3877899 (Ala234Thr) and rs7579 (a G/A base change in the 39UTR of SEPP1 mRNA) which affect plasma and lymphocyte selenoprotein activity in vivo and the relative proportion of plasma SePP isoforms.[4-8] Genetic variation in SEPP1 has been reported to be associated with several metabolic phenotypes. Two single nucleotide polymorphisms (SNPs) in SEPP1 were reported to have functional consequences on protein level and\or function. The coding SNP rs3877899 (Ala234Thr) has been shown to influence plasma selenium levels as well as plasma levels of SeP in both European Americans and South Asians.[4,9] Although the pathophysiological mechanism of gestational diabetes has not been clarified, it is considered to be linked to the anti-oxidant roles of selenium and selenoproteins levels and its gene polymorphisms. Oxidative stress is suggested to contribute to greatly increased incidence of maternal and fetal complications in gestational diabetes patients.

There is no the study searching SEPP1 gene polymorphism of GDM patients in the literature. In the present study we aimed to investigate the possible role of SEPP1 gene polymorphism in gestational diabetes as a risk factor, for the first time.

Materials and methods

Study subjects

The female subjects were examined between April 2014 and July 2016, and comprised 136 primiparous singleton pregnancies. In our planned study as a case-control study, the age range of the individuals in the groups 21-38. Gestational age was established on the basis of menstrual dates and confirmed by first trimester ultrasonography. All patients attending the clinic for the first time upon pregnancy and below 18 weeks of gestational age were recruited. Control and patients groups were included in the study according to physical examination and routine biochemical analyzes. The inclusion criteria for GDM women were a diagnosis of diabetes for the first time during the second or third trimester of pregnancy, and the exclusion criteria for GDM were women with a diagnosis of diabetes prior to pregnancy. Exclusion criteria for all subjects were tobacco use, chronic alcohol consumption, twin pregnancies, preexisting maternal chronic hypertension, preeclampsia, polyhydramnios, presence of any acute or chronic disease, liver disease, chromosomal or suspected ultrasound fetal abnormalities, maternal heart disease, and use of antihypertensive medication, preeclampsia and renal disease at the 1-year follow-up visit. Patients were followed until term to verify the fetoneonatal and maternal outcomes. All participants, patients and healthy controls were of Turkish origin, from Marmara region of Turkey. All pregnant women underwent maternal oral glucose tolerance test (OGTT) during their following visit to the clinic. Subjects were categorized into control and patients diagnosis groups, according to OGTT and physical examination results. Protocol of screening and diagnosis of GDM were adapted from the guidelines and protocols from Department of Obstetrics and Gynecology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey. All women were screened for GDM with 50 g glucose challenge test at 24th gestational week. The 50 g glucose challenge test was carried out independent of the time of day or any previous meal at about 24 weeks gestation. An oral glucose tolerance test was recommended to all patients with a 1-h test result >140 mg/dL (7.8 mmol/l). Diagnosis of GDM was established according to results of 100 g oral glucose challenge test. Patients with at least two abnormal values above the cutoff values were determined to be having an abnormal OGTT result: fasting >95 mg/dL (5.3 mmol/l); 1 h, >180 mg/dL (10.0 mmol/l); 2 h, >155 mg/dL (8.6 mmol/l); 3 h, >140

mg/dL (7.8 mmol/l).10 Healthy pregnant women had normal responses to glucose challenge test. Protocol of screening and diagnosis of GDM were adapted from the guidelines and protocols from Department of Obstetrics and Gynecology, Cerrahpasa Medical Faculty, Istanbul University, Istanbul, Turkey. All participants were informed about the survey and freely signed and dated the consent form. The protocol was approved by the Ethics Committee of Medical Faculty in Sakarya University and was conducted in accordance with the Declaration of Helsinki.

Blood collection

Medications were ceased at least 24 hours before the blood collection. Blood samples were collected in EDTA-containing tubes and plain biochemistry tubes after an overnight fasting. After immediate centrifugation (3.000xg) for 10 min at 4 oC, plasma samples were separated in Eppendorf tubes and frozen immediately at -80 oC until analysis. Routine biochemical parameters were measured by enzymatic colorimetric methods with commercially available kits (Cobas 8000, Roche Diagnostics GmbH, Mannheim, Germany).

Genotyping

Blood for DNA isolation was collected into EDTA-containing tubes and DNA was extracted from peripheral blood leukocytes using a commercial kit (Invitrogen Life Technologies Corporation, Carlsbad, CA, USA). Isolated DNA samples were stored frozen at -80°C.

Genotyping for the SEPP1 (rs3877899) gene polymorphism was performed by Allele-specific PCR (ASPCR) method. This method is a unique method used to detect single nucleotide changes in DNA. It provides a faster and more specific description than other similar methods. The method is based on binding specific primers to the region where the mutation is based. The presence of a match between the primer and the DNA template in the PCR mixture indicates whether there is a mutation. If the examined sample has mutation, amplification is positive for the mutation-specific region; if there is no mutation, the band is not visible.

The Primer pairs;

Common primer; 5' – CTTCACTTGCTGGCATATCT – 3'
Primer A (normal allele binding primer); 5' – CAACCAGGAGCACCAAAGA – 3'
Primer G (mutant allele-binding primer); 5' – CAACCAGGAGCACCAAAGG – 3'were used. These primers result in a PCR product of 121 bp. PCR mixture for SEPP1 gene is given on Table 1. Two PCR tubes were prepared for each sample. The common primer was pipetted into both tubes, primer A only into the first tube, primer G only into the second tube. The amplification product in normal homozygous individuals (AA genotype) is amplified only in the first tube (in the A tube), in the mutant homozygous individuals (GG genotype), in the amplification product only in the second tube (in the G tube), in the heterozygous individuals (the AG genotype) (Both in A and in G tube) (Table 2).

Table 1: The SEPP1(rs3877899) genetic PCR mixture (final volume 25 µl)

	Stock solution of molarity	Working solution of molarity	Final molarity
PCR Buffer	10X	____	1X
Primers C, A, Common	100 µM	10 µM	0.4 µM
dNTPs	100 mM	2 mM	0.2 mM
Taq Polymerase	5U/µM	____	1 U
DNA	____	____	~50ng
DNA	____	____	~50ng

Table 2. Bands formed after electrophoresis of the SEPP1 gene

Amplification PCR Product	Normal homozygous (AA)		Heterozygous (AG)		Mutant homozygous (GG)	
	A tube	G tube	A tube	G tube	A tube	G tube
Selenoprotein P1 (121 bç)	▬		▬	▬		▬

Amplification temperatures for the selenoprotein P1 gene; PCR conditions were as follows: initial denaturation at 95 oC for 5 min, followed by 35 cycles of denaturation at 94 oC for 30 s, annealing at 50 oC for 30 s, and elongation at 72 oC for 30 s. The final amplicon extension has been performed at 72 oC for 5 min. The amplified PCR products were separated on 3 % agarose gel in 1x Tris borate EDTA buffer followed by staining with ethidium bromide solution. The rs3877899 genotypes were identified by visualization under ultraviolet light.

Statistical analysis

SPSS Statistic 17.0 program were used for the analyses of the pa-

tients and control values. Hardy–Weinberg equilibrium was tested by Chi-square analysis. Genotype and allele frequencies were compared between cases and controls by Chi-square analysis. Odds ratio (OR) and respective 95 % confidence intervals (CIs) were reported to evaluate the effects of any difference between allelic and genotype distribution. Mann–Whitney U test and t-test were performed for the analysis of clinical characteristics and biochemical parameters. Pearson correlation test was used for the detection of the relationship between variables. A two-sided p value ≤ 0.05 was considered statistically significant.

Table 3. Clinical characteristics and biochemical parameter values of GDM and healthy pregnant groups (M±SD).

	Control Group	GDM Group	p
Weight of fetus (g)	2789.22 ± 186.25	3268.14 ± 432.86	0.001
Height of fetus (cm)	49.92 ± 0.86	50.12± 1.56	0.878
Fasting Glucose (mg/dl)	80.86±5.14	90.18±18.93	0.001
Fasting Insulin (µU/ml)	5.22±1.54	11.68±4.91	0.001
HOMA-IR	1.16±0.51	2.61±1.36	0.001
HbA1c (%)	4.67±0.51	5.31±0.37	0.001
HDL-Cholesterol (mg/dl)	62.07±12.42	62.44±13.52	0.849
LDL- Cholesterol (mg/dl)	140.82±35.86	144.31±30.99	0.489
T. Cholesterol (mg/dl)	229.35±42.29	242.48±39.22	0.033
Trygliseride (mg/dl)	195.74±75.18	199.82±73.99	0.170

Table 4. Distribution of genotypes and allele frequencies of SEPP1(rs3877899) polymorphism in patient with GDM and control groups.

Gene	GDM patients n (%)	Healthy pregnant controlsn (%)	p	OR (CI 95%)
SEPP1(rs3877899) polymorphism	72	64		
Genotypes				
AA	20 (28)	14 (24)		1
GA	31 (43)	35 (50)	0.261	1.613 (0.699-3.724)
GG	21 (29)	15 (26)	0.967	1.020 (0.394-2.643)
Alleles				
A	71 (49)	63 (49)		1
G	73(51)	65 (51)	0.989	1.003 (0.623-1.616)

Results

The clinical characteristics of subjects included in the present study are summarized in Table 3. The patient (33.96 ± 5.54) and control (34.27 ± 4.37) groups were similar in age (M±SD). We found that weight of fetus, HbA1c, fasting insulin, HOMA-IR, fasting glucose and total cholesterol levels were higher in gestational diabetic pregnants compared to healthy pregnant women (p=0.001, p=0.001, p=0.001, p=0.001, p=0.033, respectively) HbA1c showed a positive correlation with fasting insulin, fasting glucose and HOMA-IR in GDM group (r=0.515, p=0.01; r=0.331, p=0.01; r=0.591, p=0.01, respectively). Total cholesterol also showed a positive correlation with LDL-cholesterol and triglycerides (r=0.904, p=0.01; r=0.533, p=0.01, respectively). The SEPP1 (rs3877899) gene polymorphism was successfully genotyped in 72 women with GDM and 64 control subjects. Frequencies of SEPP1 (rs3877899) genotypes and alleles observed in patients with GDM and pregnant healthy women are shown in Table 4. The frequencies of the AA, GA and GG genotypes were found as 28 %, 43 % and 29 % in pregnant women with GDM and 24 %, 50 % and 26 % in healthy pregnant women, respectively. Our results indicated that the distribution of the SEPP1 genotypes and alleles did not differ significantly among subjects with or without GDM (p>0.05).

Discussion

Although GDM is related to metabolic conditions, recent studies have suggested that there may be various environmental and genetic risk factors affecting the susceptibility for GDM, in which molecular mechanisms remain unclear. Selenoprotein P is a hepatokine and the role of hepatokines is unclear in the pathophysiology of GDM. Recent studies reported that the some hepatokines, such as selenoprotein P, fibroblast growth factor-21 and fetuin-A were associated with the pathophysiology of GDM, even though their circulating levels were not changed.[11-13] Selenoprotein P (in humans encoded by the SEPP1 gene) is a hepatokine mainly produced by the liver and serves as selenium supply protein in selenium homeostasis. In the literature, there is no study investigating the relationship between selenoprotein gene polymorphism and gestational diabetes mellitus risk. In the present study, we investigated the SEPP1 gene polymorphisms and the risk of GDM. We found that SEPP1 polymorphism did not alter the risk of GDM in our population. However, we found that weight of fetus, HbA1c, fasting insulin, HOMA-IR, fasting glucose and total cholesterol le-

vels were higher in GDM pregnancies compared to healthy pregnant women.

Single nucleotide polymorphism (SNP) has been shown to affect selenium and SeP expression levels, in vivo. Selenoprotein synthesis is highly dependent on Se intake with nutrient, and therefore the effect of the SNPs described herein should be modified by Se intake. For that reason, in future studies, determination of the genotype for selenoprotein SNPs should be combined with the measurement of the Se status. These interactions between gene polymorphism and diatery intake existing in the selenium metabolism should be researched as a risk factor of the metabolic diseases and GDM.

It has been shown that selenoprotein P induced insulin resistance and hyperglycemia by disrupting the insulin signaling. Additionally, SeP functions as a both Se transport and antioxidant. There are conflicting results associated with changes in selenoprotein levels in type 2 DM. The role of hepatokines in the pathogenesis of gestational diabetes has not been fully elucidated. However, recent studies have suggested that changes in selenoprotein P levels in type 2 diabetes mellitus may be associated with insulin resistance, diabetic complications, and inflammation.[14-16] In the literature, there is a limited number of studies investigating selenoprotein P gene polymorphisms despite a large number of studies on the circulating selenoprotein P and mRNA levels. SEPP1 gene polymorphism has been studied in colorectal, breast and prostate cancer. Most of these stuies are related with its antioxidant properties. rs3877899 in SEPP1 modulates selenoprotein concentrations and activities in plasma, erythrocytes and lymphocytes and plasma SePP isoforms, suggesting that these polymorphisms may affect Se transport in tissues. Meplan et al. suggested that rs3877899 (SEPP1) genotype was related with the risk of breast cancer.[17] Another study found that rs3877899 (SEPP1) is a risk of prostate cancer, another hormone-dependent cancer.[18] In our study, we found that the frequencies of the AA, GA and GG genotypes in the pregnant women with GDM were same as healthy pregnancy rates. Distribution of the SEPP1 genotypes and alleles did not differ significantly among subjects with or without GDM. It should be kept in mind that one of the major limitations to our study was the small sample size, which may have influenced the statistical power of our analyses. Another limitation of our study was selection of patients from same center. It should be clarified in further studies with larger population selected from different centers. In addition, our study was not designed to investigate the association between SEPP1 gene polymorphism and plasma/serum SeP and selenium levels in the mothers and their offspirings.

Although SeP plays a key role in glucose metabolism and the regulation of insulin sensitivity, the SEPP1 polymorphism did not changed occurrence of GDM in our population. Different mechanisms may be involved in etiopathogenesis of GDM. However, it should be clarified with further studies in larger populations. SEPP1 (rs3877899) polymorphism has no role in development of gestational diabetes in Turkish women.

Conflict of interest The authors declare that they have no conflicts of interest related to the publication of this manuscript.

References

1. Metzger BE, Coustan DR. Summary and recommendations of the fourth international workshop-conference on gestational diabetes mellitus. The Organizing Committee. Diabetes Care 1998; 21: B161–7.
2. Catalano PM, Kirwan JP, Haugel-de Mouzon S, King J. Gestational diabetes and insulin resistance: role in short- and long-term implications for mother and fetus. J Nutr 2003; 133: 1674S–83S.
3. Yan M, Mehta JL, Zhang W, Hu C. LOX-1, oxidative stress and inflammation: a novel mechanism for diabetic cardiovascular complications. Cardiovasc Drugs Ther 2011; 25(5): 451–59.
4. Meplan C, Crosley LK, Nicol F, Beckett GJ, et al. Genetic polymorphisms in the human selenoprotein P gene determine the response of selenoprotein markers to selenium supplementation in a gender-specific manner (the SELGEN study). Faseb J 2007; 21: 3063–74.
5. Meplan C, Nicol F, Burtle BT, Crosley LK, et al. Relative abundance of selenoprotein P isoforms in human plasma depends on genotype, Se intake, and cancer status. Antioxid Redox Signal 2009; 11: 2631–40.
6. Cooper ML, Adami HO, Gronberg H, Wiklund F, Green FR, et al. Interaction between single nucleotide polymorphisms in selenoprotein P and mitochondrial superoxide dismutase determines prostate cancer risk. Cancer Res 2008; 68: 10171–177.
7. Meplan C, Hughes DJ, Pardini B, Naccarati A, et al. Genetic variants in selenoprotein genes increase risk of colorectal cancer. Carcinogenesis 2010; 31: 1074–1079.
8. Steinbrecher A, Meplan C, Hesketh J, Schomburg L, et al. Effects of selenium status and polymorphisms in selenoprotein genes on prostate cancer risk in a prospective study of European men. Cancer Epidemiol Biomarkers Prev 2010; 19: 2958–68.
9. Karunasinghe N, Han DY, Zhu S, Yu J, et al. Serum selenium and single-nucleotide polymorphisms in genes for selenoproteins: relationship to markers of oxidative stress in men from Auckland, New Zealand. Genes Nutr. 2012; 7: 179–90.
10. Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. Am J Obstet Gynecol 1982; 144:768–73.
11. Altinova AE, Iyidir OT, Ozkan C, et al. Selenoprotein P is not elevated in gestational diabetes mellitus. Gynecol Endocrinol. 2015; 31(11): 874-6.
12. Farhan S, Handisurya A, Todoric J, et al. Fetuin-A characteristics during and after pregnancy: result from a case control pilot study. Int J Endocrinol 2012; 2012: 896736.
13. Stein S, Stepan H, Kratzsch J, et al. Serum fibroblast growth factor 21 levels in gestational diabetes mellitus in relation to insulin resistance and dyslipidemia. Metabolism 2010; 59: 33–7.
14. Misu H, Takamura T, Takayama H, et al. A liver-derived secretory protein, selenoprotein P, causes insulin resistance. Cell Metab 2010; 12: 483–95.
15. Yang SJ, Hwang SY, Choi HY, et al. Serum selenoprotein P levels in patients with type 2 diabetes and prediabetes: implications for insulin resistance, inflammation, and atherosclerosis. J Clin Endocrinol Metab 2011; 96: E1325–9.
16. Roman M, Lapolla A, Jitaru P, et al. Plasma selenoproteins concentrations in type 2 diabetes mellitus – a pilot study. Transl Res 2010; 156: 242–50.
17. Meplan C, Dragsted LO, Ravn-Haren G, et al. Association between polymorphisms in glutathione peroxidase and selenoprotein P genes, glutathione peroxidase activity, HRT use and breast cancer risk. PLoS One. 2013;10; 8(9):e73316.
18. Cooper ML, Adami HO, Gronberg H, Wiklund F, Green FR, et al. (2008) Interaction between single nucleotide polymorphisms in selenoprotein P and mitochondrial superoxide dismutase determines prostate cancer risk. Cancer Res 2008; 68: 10171–77.