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Original Article

Tumor necrosis factor alpha promoter polymorphism studies in pregnant women

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ABSTRACT

Aims: The aim of this study was to explore the possible association between the −850 C/T polymorphism in the tumor necrosis factor alpha (TNF-α) gene promoter, and pregnancy-associated diseases such as gestational diabetes mellitus (GDM) and preeclampsia (PE), in south Indian women. GDM and PE are common complications that occur during pregnancy and are the leading causes of perinatal mortality. To date, the mechanisms that initiate GDM and PE in humans have remained elusive.

Methods: This prospective case-control study was carried out with 505 pregnant women: 140 women had GDM, and 105 with PE. Remaining 260 women were age- and frequency-matched controls. TNF-α (−C850T) genotyping was determined by polymerase chain reaction with restriction fragment length polymorphism (PCR-RFLP) analysis.

Result: We found no statistically significant difference in the genotypic and allelic distribution between GDM women and controls (for CT + TT vs. CC, $\chi^2 = 0.3919$; $p = 0.61$; Odds Ratio (OR) = 0.76 (95% CI: 0.203–1.876)). No significant differences was observed in the allele and genotype frequency between PE women and controls (for CT + TT vs. CC, $p = 0.31$; OR = 0.55 (95% CI: 0.171–1.784); T vs. C, $p = 0.71$; OR = 0.94 (95% CI: 0.680–1.3)).

Conclusion: From our results, we conclude that the (−C850T) promoter polymorphism has no role in the propensity of pregnant women from south Indian populations to develop GDM or PE.

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1. Introduction

Gestational Diabetes Mellitus (GDM) and preeclampsia (PE) are multifactorial and severe, complicated disorders of human pregnancy, which cause maternal, as well as perinatal morbidity and mortality.[1] GDM and PE are relatively common diseases and affect ~16% of all pregnancies, resulting in a variety of complications that primarily affect the fetus, including macrosomia, stillbirth, jaundice, and respiratory distress syndrome. The relationships and genetic background predisposing pregnant women to GDM and PE are still not clear.[2–4] GDM is characterized by a carbohydrate intolerance of variable severity that develops during pregnancy when the pregnancy hormones interfere with the body's ability to use insulin, the hormone that modulates glucose uptake into cells. As a result, glucose levels in the bloodstream become elevated.[5] PE is a hypertensive disorder associated with elevated blood pressure and proteinuria that frequently develops after 20 weeks of gestation.[6] The common risk factors for GDM and PE include obesity, increased maternal age, a history of GDM/PE, previous adverse pregnancy outcomes, and a high-risk ethnicity. Both the diseases are serious threats to the mother and the child.

Single-nucleotide polymorphisms (SNPs) in the tumor necrosis factor alpha (TNF-α) promoter gene can regulate plasma levels of TNF-α, and the action of insulin.[7,8] However, these findings are not supported by some studies.[9,10] Kato et al[11] first reported a base substitution of C→T at position −850 of the promoter region of human TNF-α in 1999. TNF-α is a multifunctional cytokine produced mainly by macrophages and lymphocytes, as well as in pregnancy by trophoblast cells. It is a pro-inflammatory cytokine involved in the pathogenesis of various autoimmune and inflammatory diseases,[12] and has been implicated in mediating insulin resistance.[13] The polymorphisms in the promoter region are found at positions −1032, −863, −857, −850, −575, −375, −308, −274, −243, −237 and −162.[14–16] There are two promoter transition polymorphisms (−G308A and −C850T) that have been associated with chronic inflammatory diseases such as ulcerative colitis, rheumatoid arthritis, PE, and Crohn's disease.[17] One such SNP, the −805 C/T polymorphism, is located at position 805 in the promoter region of the TNF-α gene and has been selected for this study. It was recently suggested that TNF-α may play an important role in apoptosis and obesity-related metabolic disorders such as insulin resistance, disturbance of lipid metabolism, and hypercoagulability, and may be one of the mediators of atherosclerosis.[18] TNF-α therefore may play an important role in the pathophysiology of multifactorial diseases like GDM and PE.

Previously, investigators have focused on several new potential mediators of insulin resistance, which have a key role in the development of GDM, including the cytokine (TNF-α).[19,20] There is a lot of evidence that suggests a relationship between PE and TNF-α. Increased serum TNF-α activity has been identified in PE.[21] The main objective of this case-control study was to identify the association between TNF-α (−850C > T) gene promoter polymorphism (rs1799724) and south Indian pregnant women who develop GDM and PE.

2. Materials and methods

2.1. Pregnant women study

A case-control study was conducted in 505 pregnant women from the south Indian population; this included patients diagnosed with GDM ($n = 140$) and hypertension (PE) ($n = 105$) during pregnancy. All control pregnant women ($n = 260$) were recruited from the same demographic area based upon age and normal glucose, systolic blood pressure (SBP) and diastolic blood pressure (DBP) values. This study was carried out in the Kamineni Hospitals, Hyderabad, India. The exclusion criteria for GDM were, women with a diagnosis of diabetes prior to pregnancy, and for PE women, those with chronic hypertension. The study was approved by the Ethics Committee of Kamineni Hospital, Hyderabad. Written informed consent was obtained from all the pregnant women who participated in this study.

2.2. Validation of GDM women

GDM subjects were selected based on the results of glucose tests. All the pregnant women were screened for GDM between 24 and 28 weeks of gestation, as per the American Diabetes Association (ADA) guidelines.[22] A glucose challenge test (GCT) was performed by administering 50 g of glucose to pregnant women whose fasting plasma glucose value exceeded 130 mg/dL. The GCT positive women underwent a standard oral glucose tolerance test (OGTT) involving the administration of 100 g of glucose after overnight fasting and three days of unrestricted diet. Blood samples were drawn during fasting, 1, 2, and 3 h after glucose administration. In this study, women were classified as GDM when two or more glucose values met or exceeded the threshold values that were established in our earlier studies.[23] All women with GDM were monitored for metabolic and obstetric manifestations and the need for either a supplementary treatment with insulin or for a controlled diet until delivery.[24]

2.3. Diagnostic criteria for PE women

PE was diagnosed based on two consecutive measurements of systolic and diastolic blood pressure taken, after the 20th week of pregnancy, at least 6 h apart. Increase in diastolic blood pressure to >110 mmHg or a rise of 15–30 mmHg above the normal pre-pregnancy values indicates PE. This could be accompanied with 300 mg protein in the 24 h urine specimen or urine dipstick >1+.[25,26]

2.4. DNA isolation and genotyping

For confirmation of the disease, serum from blood (3 mL) was collected from the GDM women (and from the control women, for comparison), and 2 ml of blood sample collected in EDTA vial was used to extract genomic DNA following salting out method.[23] The DNA was stored at −20 °C until processed. TNF-α genotypes were identified by polymerase chain reaction, followed by restriction fragment length polymorphism (PCR-RFLP) analysis, as previously described for the −850C > T