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RESEARCH ARTICLE – GENERAL BIOCHEMISTRY, GENETIC AND MOLECULAR BIOLOGY

Functional role of TGFβ1 -509C/T gene polymorphism in Susceptibility to preeclampsia in pregnant women

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Abstract

Background: Preeclampsia [PE] is a genetic and vascular disorder that occurs during pregnancy. It has multiple symptoms, including high blood pressure and protein in the urine. This disease results from poor blood supply to the placenta.

Objective of study: The aim of the present study was to investigate the association between TGFβl promoter polymorphism -509C/T and PE in South Indian women. A candidate factor in the development of this disease is the immunoprotein (transforming growth factor β1 cytokine).

Materials and Methods: In this study, a total of 100 pregnant women were screened for functional polymorphisms of the TGFB1 gene [C-509 T], including 50 patients with PCS and 50 stable controls. While homozygous CC constituted 76% of PCS cases and 70% of normal healthy pregnant women, 24% of PCS cases and 30% of controls were heterozygous CT.

Results: The results showed that there were no significant differences. Statistically significant differences between preeclampsia cases and controls at this polymorphic site in genotype distribution and allele frequency. Molecular defects in this immunoprotein may lead to a defect in the regulation of the placental blood vessels, which affects the occurrence of apoptosis in target cells, and may also act as a major controller of the Th1/Th2 cytokine balance and the development of peripheral anti-inflammatory T cells [FOXP3 + Tregs].

Conclusion: In conclusion, preeclampsia cannot be associated with polymorphisms in the TGF-beta1 promoter region at position -509 (C/T). In pregnant women, the clear urine that persists is produced by high TGF-b1 levels, and the function of the cells (glomerular cells) is responsible for raising TGF-β1 levels.

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Keywords: TGFβ1 -509C/T Gene, Preeclampsia, Gene Polymorphism, Hypertension, Proteinuria.

1. Introduction

After five months of pregnancy, some women may show some symptoms that may be delayed, mainly high blood pressure and the appearance of urine to cover preeclampsia. The rate ranges from 2-8%, and the third is such a small, severe case in human pregnancy. This disease is considered one of the most important causes of death in pregnant women before birth, and this is at the level of advanced diseases. Although we do not understand the cause of the disease, it is difficult to determine the main cause due to preeclampsia [1]. Preeclampsia affects approximately 5-10% [1] every year in pregnancies worldwide; some studies have classified preeclampsia as the second cause of morbidity and mortality in pregnant women as well as newborns [2]. When diagnosing preeclampsia due to the presence of some symptoms, namely high blood pressure and the appearance of urine recording in the pregnant mother after the fifth month of pregnancy, the woman who ruled in the first four months had normal blood pressure [3, 4].

Symptoms of the disease can be variable, as mild hypertension with protein in the skin is diagnosed severely, persistent hypertension and protein in the urine may affect the health and life of the mother, as seizures and damage to the mother's external organs may occur. Many studies or evidence have indicated that many factors cause preeclampsia, so it is a disordered disorder that may include the solidarity of several negative and environmental stressors [5]. It has been proven that the multiplicity and view of the TGFB1 gene, which is located on human chromosome 19 (19q13.1–13.3) [6], may have a partial role in the emergence of the disease through estimates and begins for the protein (cytokine) produced by this gene [7-9]. Many single nucleotide polymorphisms (SNPs) have been identified in this TGFB1 gene, but there is a single nucleotide polymorphism (SNP) that gained function and transferred C>T to position 509 before the start site in the TGFB1 gene. This polymorphism is

specific to the activator region of the transcription factor differentially stimulating TGFB1 and TGF-b1 levels in serum [10]. The latter polymorphism appears at position 29 of the translated protein, resulting in a leucine to proline substitution at position 10 of an empty peptide of TGF-b1, and has been confirmed to affect TGF-b1 production in HeLa cells. Conflicting results have subsequently emerged regarding the lack of connection between the TGFB1 gene and PE [11–18]. In light of this, we attempted to explore the compatibility between the TGFB1 C-509T polymorphism and PE predisposition.

2. Materials and method

One hundred participants were gathered for this case-control study, including fifty pre-eclampsia subjects and fifty stable pregnant women (control participants) who visited Gandhi Hospital and the Government Maternity Hospital in Hyderabad, India, between 2013 and 2014. The Institutional Ethical Review Board of Osmania University in Hyderabad, India, authorized the protocol's use in the study, and each participant gave their written informed consent. Sterile EDTA vacutainers were used to collect blood samples, and a questionnaire asking about clinical, demographic, and family history information was given to each participant in the current investigation. For future research, 3 ml of blood is drawn from patients and controls and kept at 4°C. The selected subjects provided blood samples for genotyping analysis.

2.1. Selection requirements for PE patients and healthy pregnant women Inclusion criteria

Preeclampsia is described by the International Society for the Study of Pregnancy Hypertension as hypertension with systolic blood pressure > 140 mm Hg and diastolic blood pressure > 90 mm Hg after 20 weeks of gestation with proteinuria> 300 mg in 24-hour collection or > 1 + in dipstick tests not associated with urinary tract infection or ruptured membranes.

2.2. Exclusion criteria

The current research excludes women with chronic hypertension, diabetes, polycystic ovary syndrome or index pregnancy with multifetal conception or born by assisted reproductive technology or with early rupture of the membranes or unexplained menstrual bleeding or antihypertensive medication use.

2.3. Control subjects

Healthy pregnant volunteer women (with pregnancy stage match) with normal hypertension (<140/90 mm Hg) and with normal proteinuria range (<300 mg in a 24-hour collection or < 1+ on dipstick testing) are considered as control subjects in the present study. Information is obtained from both patients and normal pregnancy stage-matched control subjects, regarding their age, age of menarche, obstetric details, social, habits, educational status, age at first conception, age at the conception of mother/mother-in-law, previous medical history, family history in the form of three generations.

2.4. Molecular analyses

Five milliliters of peripheral venous blood were obtained and preserved at 4 ° C in EDTA-coated vials. Genomic DNA was derived using the salting method [19] from whole blood. Spectrophotometric absorbance measurements at 260 nm and 280 nm, which were further confirmed by 1% agarose gel electrophoresis, determined DNA purity and concentration. As per previously published protocols, genotyping of the SNPs in the TGFB1 gene, namely the C-509 T in the promoter region, was performed using PCR-restriction fragment length polymorphism (RFLP), with slight modifications [20]. The C-509 T SNP PCR amplification was conducted at a cumulative volume of 10 µl with a PCR master mix containing: 100 ng of genomic DNA, 1.25 µl of Taq buffer, 0.3 µl of dNTP mix, 0.15 µl of forward (5-CAGACTCTAGAGAGACTGTCAG 3-) and reverse (5-GTCACCAGAAAGAGAGGAC 3-) primers (Bioserve Biotechnologies, Hyderabad, India) and 0.3 µl of Taq DNA polymerase (Labpro, Hyderabad, India). The PCR conditions were as follows: 95 ° C for 5 minutes; 35 95 ° C intervals for 30 s, 58.4 ° C for 45 s and 72 ° C for 30 s; and a final extension for 10 minutes at 72 ° C. The PCR product was digested with endonuclease (Labpro) restriction (Eco81I) at a final concentration of 0.25 U / l for 2 h. At 37 ° C in a dry bath and subsequently electrophoresed at 100 V on a 2 % agarose gel, visualized by ethidium bromide staining and examined by gel doc (Bio-Rad) under UV light. The existence of the 418-bp band, heterozygous CT genotype by the existence of 418-bp, 228-bp, 190-bp bands and homozygous TT genotype by the presence of the 228-bp, 190-bp bands was detected by the CC genotype Fig. 1.

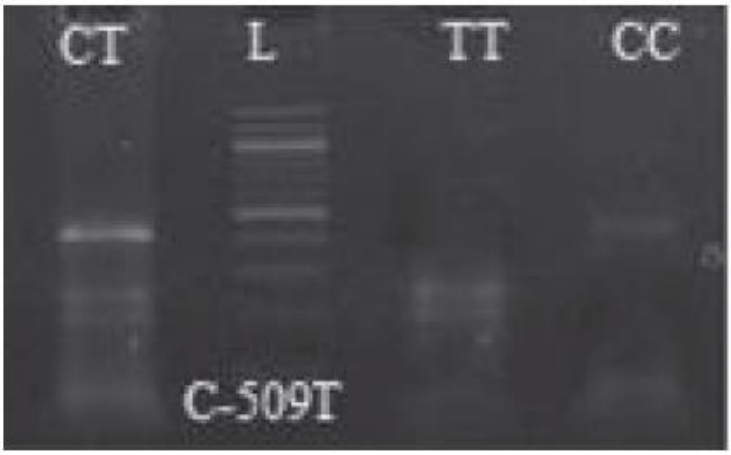

Fig. 1. TGFB1 C-509 T polymorphism representative gel photograph

3. Results

Table 1 summarizes the differences in general characteristics between the two main research groups (patients and controls). The mean systolic and diastolic blood pressure and mean gestational age differed considerably between the two groups. The mean sample age, mean gestational duration and mild and extreme subgroups did not differ significantly between the two PE patient groups and the control groups (P > 0.0001).

Table 1. Clinical and Demographic Characteristics of Pre-eclampsia Patients and Normal Pregnant women

characetristics	**(n=50)patients**	**(n=50)controls**	***P value***
age at sample collection	22.5± 3.21	22.6± 2.89	0.0001<
gestational age	30.2 ±5.34	36.02± 0.96	0.0001 <
Systolic blood pressure (mmHg)	152.1± 12.5	116.6± 5.14	0.0001<
Diastolic blood pressure (mmHg)	101.2±9.30	78± 4.04	0.0001<
Proteinuria (mg/dL)	3.16± 0.96	-	0.0001<
Pregnancy complications (IUD& IUGR	15 (30%)	-	0.0001<
Recurrence cases	10 (20%)	-	0.0001<

Presents the allele and genotype frequencies of the SNPs under analysis, namely TGFB1 C-509 T. For SNPs, genotype proportions in controls and PE patients were not compatible with HWE (P < 0.05). TGFB1 C-509 T's allele frequencies did not differ between the PE and control classes, although there was considerable difference in the genotype frequencies. Aincreased occurrence of the CC genotype with a concomitantly reduced frequency of the TT genotype was seen in the patient population Table 2, Fig. 2.

Table 2. Showing the distribution of genotypes, allele frequencies and odds ratio for the TGF-βl – 509C/T polymorphism in patients and controls.

Polymorphism	**Genotypes**	**Controls**	**Patients**	**Allele frequencies**	**Controls**	**Patients**	**X^2 P value**
TGFβ1 -509C/T	CC	35(70%)	38(76%)	C	0.18	0.88	1.412 0.05 >P
	CT	15(30%)	12(24%)	T	0.12	0.82	0.45 0.05 >P
	TT	0	0				

Relevant OR under the over-dominant and recessive genetic models (P < 0.05) was observed for the strength of interaction of TGFB1 promoter polymorphism with PE, which was defined by comparing genotype contrasts between patients and controls table 3.

Table 3. Odds ratios for comparison of genotypes contrasts in patients and controls

Polymorphism	**Model**	**Genotypes**	**OR (95%CI)[a]**	**P value**
TGFβ1-509C/T	Dominant	CC	1.35	> 0.05
		CT+TT	0.21(0.55-3.29)	
	Recessive	CC+CT	1.00	> 0.05
		TT	0.73(0.30-1.78)	
	Over dominant	CC+TT	1.00	> 0.05
		CT	0.13(0.04-0.44)	

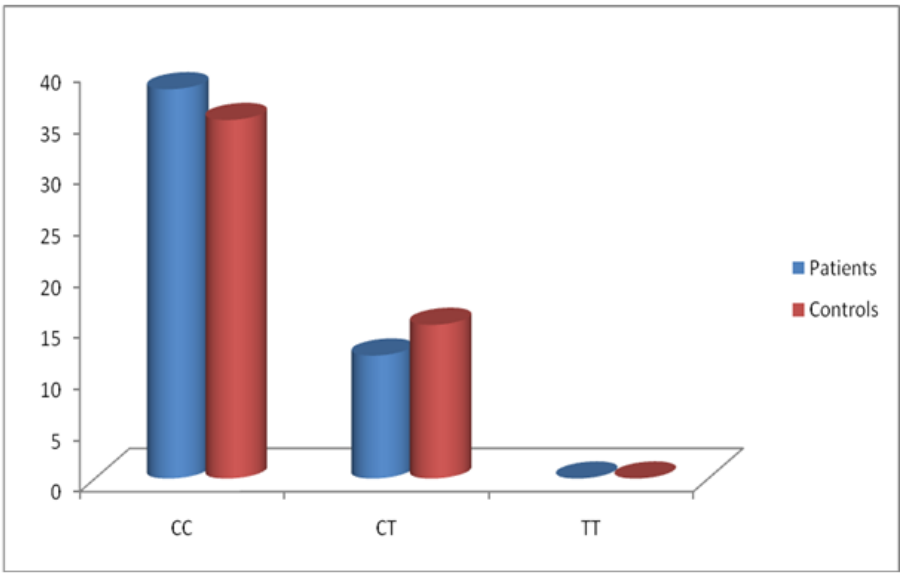

Fig. 2. Showing the distribution of genotypes of TGF-β1 -509 C/T polymorphism

4. Discussion

The aim of the present study was to investigate the association between TGFβl promoter polymorphism -509C/T and PE in South Indian women. Few studies have discussed this topic regarding the affinity of TGFB1 promoters and susceptibility to PE worldwide. Some of the results observed no association between these polymorphisms and preeclampsia, while others suggested an association between their contribution and preeclampsia susceptibility [21- 29]. The frequencies of polymorphic alleles in preeclampsia patients are consistent with previous studies [30-34]. The results obtained in our study show that there is a significant difference in the frequencies of polymorphic or C-509T between preeclampsia patients and healthy pregnant women in contrast to previous reports. The difference may be small for some time, as the disparity may be due to the smaller eye size and ethnic background of the study population [35,36]. Regarding the effects of the C-509T polymorphism, CT exerted a very significant effect. An important explanation for this result can be made based on previous reports that the TGFB1 C-509T polymorphism affected serum TGF-b1 levels, where it was shown that the T allele and the TT type have a significant relationship and mRNA expression and contribute to serum TGF-b1 levels. Accordingly, its effect could be high CT, which will act on the level of serum TGF-b1 protein production, which is intermediate, which is used for proper walking and the formation of blood vessels and adipose tissue. To prove this hypothesis, studies show that the level of TGF-b1 in plasma is not constant during the differential differences, accordingly it can be adopted accordingly, which affects the increased or decreased expression of TGF-b1 and may be harmful to normal pregnancy. The fantasy of heterogeneity in protest may be suitable for the appropriate gynecological Democrats according to the different stages of pregnancy. On the other hand, the TT mutation of the C-509T polymorphism appeared to show a 14-fold increased risk of PE, although the risk was not as significant as the protective effect and had no significant effect on the protective effect it provided against CT [23,24]. There is a high-production version of the "T" allele that aims to raise the plasma TGF-b1 level, leading to endothelial cell function disturbances through upregulation of endothelin-1. In light of what was observed by the awards ceremony, plasma TGF-b1 concentrations in women with PE were higher than in pregnant women. In addition, high TGF-b1 levels led to an increase in angiotensin II levels and stimulated renal production by the juxtaglomerular cells with hypertension [37].

5. Conclusion

The preeclampsia cannot be associated with polymorphisms in the TGF-beta1 promoter region at position -509 (C/T). In pregnant women, the clear urine that persists is produced by high TGF-b1 levels, and the function of the cells (glomerular cells) is responsible for raising TGF-β1 levels.

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Nomenclature & Symbols			
PE	Pre-eclampsia	SNPs	Single Nucleotide Polymorphisms
TGFB1	Transforming Growth Factor-β1	RFLP	Restriction Fragment Length Polymorphism
C	Cytosine	T	Thymine
EDTA	Ethylenediaminetetraacetic acid	UV light	Ultraviolet radiation

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