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TNF-α AND ITS ASSOCIATION WITH NITRIC OXIDE SYNTHASE GENE IN PREECLAMPSIA

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ABSTRACT

Pathophysiological processes in preeclampsia (PE) are influenced by genetic factors. To better characterize the host genetic factors determining the susceptibility to PE, we evaluated the influence of two polymorphisms in the NOS2A gene on the risk of developing PE and correlated with TNF- α. The polymorphisms were studied by DNA sequencing and the levels of TNF- α were measured by high sensitivity enzyme-linked immunosorbant assay. The study subjects were 30 pre-eclamptic women compared by age matched 30 healthy pregnant women. Kruskal-Wallis non-parametric analyses of variance followed by Mann-Whitney U-test were used for statistical analysis. Both (G300A Exon8 and G274TExon16) single nucleotide polymorphism (SNP) showed statistically significant association with preeclampsia ($p< 0.05$). The levels of TNF- α in pre-eclamptic subject were increased significantly when compared with the healthy controls ($P < 0.001$). Higher TNF- α level was observed in TT genotypes of G274T Exon16 in comparison to GG or GT genotypes in preeclamptic patients.These results indicate the relationship between increased TNF- α levels and NOS2A polymorphism leading to its altered expression in preeclamptic patients.

KEY WORDS

Cytokine, Nitric Oxide Synthase, TNF- α, Preeclampsia

INTRODUCTION

Preeclampsia is a human pregnancy-specific disorder that is diagnosed by the new appearance of hypertension and proteinuria after 20 weeks' gestation. It can present as asymptomatic hypertension and proteinuria at one end of the spectrum to multi-organ failure at the other. The precise origins of the disease remain enigmatic, but the placenta undoubtedly plays a role, since delivery inevitably lead to rapid recovery. Consequently, a large proportion of the perinatal morbidity is due to iatrogenic prematurity. It is estimated that up to 15% of

preterm births are secondary to delivery for preeclampsia[1].

TNF- α is implicated in the inhibition of endovascular trophoblast invasion[2] and in promoting endothelial cell activation[3], the characteristic placental and systemic vascular pathology of preeclampsia. Other reports indicate that interleukin (IL)-1beta, which also induces MCP-1 expression, is involved in the genesis of preeclampsia as well. Thus, TNF- α and IL-1b are potential mediators of physiological and pathological MCP-1- mediated decidual macrophage infiltration[4].

Human pregnancy has been shown to induce expression of iNOS in smooth muscle myocytes[5] and blood vessel endothelial cells. There are diverse ways of induction, each being specific for a certain cell type or tissue. Cytokines and pressure are two of the recognized inducers of transcription, exerting their effect through an intricate network of transcription factors[6]. Numerous cytokine responsive elements have been found in the promoter and regulatory region of iNOS, with a number of them residing in the region upstream of -4 kb from the transcriptional start site[7, 8]. This suggests that iNOS may play a role in the regulation of myometrial tone during normal human pregnancy[5].

Thus, despite being one of the leading causes of maternal death and a major contributor of maternal and perinatal morbidity, the mechanisms responsible for the etiopathogenesis of preeclampsia are still unclear. Hence, this study was designed with the aim to provide a better understanding of the potential etiopathogical factors of preeclampsia.

MATERIAL AND METHOD

The study protocol was approved by Lady Hardinge Medical College's Ethical Committee. This case-control study consisted of 30 preeclamptic patients and 30 healthy pregnant controls. The diagnosis of preeclampsia was made by strict criteria: BP ≥140/90 mmHg after 20 weeks gestation and Proteinuria ≥ 300 mg / 24 hr or ≥ 1+dipstick.

Tumor necrosis factor- α in serum was determined by solid phase sandwich Enzyme Linked – Immuno-Sorbent Assay with sensitivity less than 10 pg/mL using kits obtained from DIACLONE Research, France. All assays were conducted according to manufacturer's protocols. These experiments were performed in duplicate, and the amount of TNF-α in each sample was determined by extrapolating absorbance values to TNF-α concentrations using the standard curve.

Genomic DNA was extracted from peripheral blood using a standard salting-out protocol[9]. PCR amplifications were carried out for exon 8 and 16 of NOS2A gene individually. All the PCR amplification reactions used 50 ng of the template DNA, 1.5 mM MgCl2, 20.0 picomole each of the forward and reverse primers, 0.2 mM dNTP (Amersham, UK), 1.5units of Taq polymerase (Bangalore Genei, India) in 50 µl of reaction. The purified PCR products were used as templates for cycle sequencing reaction based on dideoxy termination reaction. 35-50 ng of the purified PCR product was amplified using 1 pM of the forward or reverse primer and 4.0 µl of ready reaction mix in a 10 µl reaction. Samples were loaded on capillary-based AB1 3730 automated DNA sequencer (Applied BioSystems, California, USA) as per the standard procedures. Sequence data obtained was analyzed using Basic Local alignment Search Tool (htpp://www.ncbi.nlm.nih.gov/BLAST/) and Seqman software of DNA star.

Statistical Analysis

Statistical analysis between the groups was determined by the Kruskal Wallis test and Mann-Whitney U-test. $P<0.05$ was considered statistically significant.

RESULTS

Serum TNF- α levels were significantly elevated in PE patients in comparison to normal controls (p=0.001) (fig1).Serum NO levels were significantly elevated in PE patients

(36.5+12.7uM/L in pts vs 23.7+6.3uM/L in controls,p=0.00). G300A Exon8 (p=0.02, OR=2.3, 1.13<OR<4.8) and G274T Exon16 (p=0.001, OR=2.50, 1.40<OR<4.4) single nucleotide polymorphism were found to be statistically significant. There was positive correlation between TNF- α levels and TT genotypes of G274T Exon16, though the p-values were not statistically significant. (Table 1& Fig 1)

Table 1
Correlation between TNF & NO and Studied SNPs

	G300A exon8		G274T exon16	
	TNF	NO	TNF	NO
Chi-Square	0.689	2.563	0.093	1.31
Df	2	2	2	2
Asymp. Sig.	0.708	0.278	0.955	0.52

a Kruskal Wallis Test , b Grouping Variable: G300A exon8 / G274T exon16, c GROUP = CASE

Fig 1
NO & TNF levels in G300A exon8 and G274T exon16 genotypes.

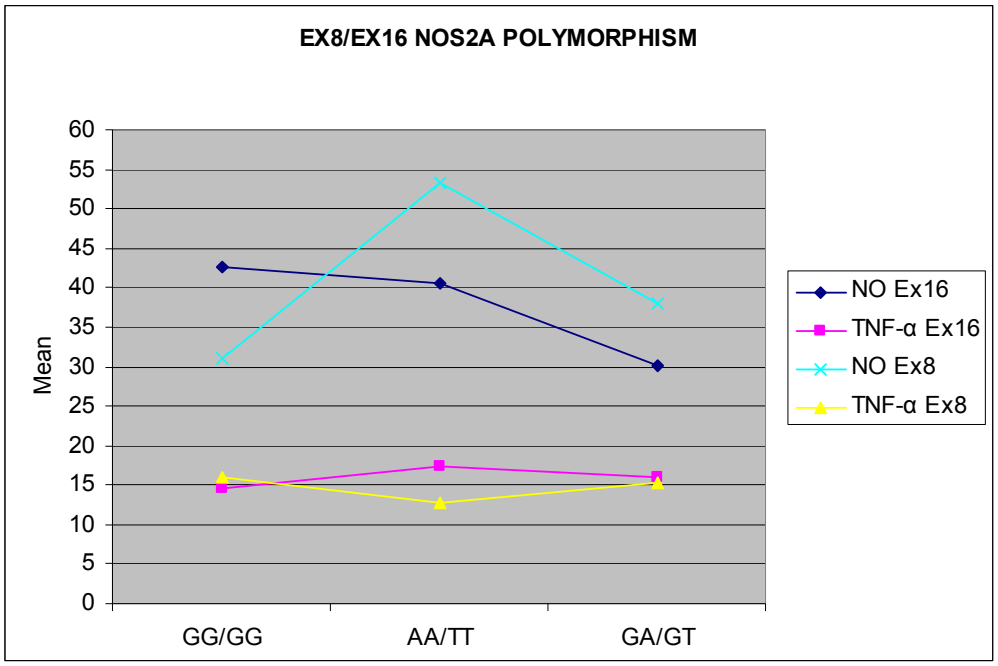

Figure depicts comparison between G300A Exon8 and G274T Exon16 genotypes on x-axis with mean serum NO and TNF levels on y-axis respectively

DISCUSSION

PE is a hypertensive disorder of pregnancy, with genetic factors believed to play a significant role in susceptibility. In the present study, we investigated single nucleotide polymorphism in the iNOS gene G300A exon 8 and G274T exon16 in patients with preeclampsia and compared it with the genotypes of healthy pregnant controls. This

study indicated statistically significant association of the tested iNOS polymorphism in an Indian population. Various studies have reported significantly lower levels of iNOS mRNA in the placental villous tissue and myometrium from women with preeclampsia[10,11] iNOS is primarily expressed in macrophages present in villous stroma throughout gestation in both normal and preeclamptic placentas[11], suggesting that iNOS might participate in the formation of an immune barrier against maternal insult[11].

The TNF-α levels were found to be significantly higher in preeclampsia in comparison to control group (PE 20.43 ± 12.16pg/ml vs control 9.71 ± 6.34pg/ml; p=0.001). This is in line with results from various previous report from Muzammil S and especially with a report from Peracoli JC where TNF- α levels were high in PE subjects than controls and decreased in puerperium[12,13].

It was also observed that AA genotype of G300A exon8 SNP showed higher serum NO levels in comparison of homozygotes for G or A alleles. Similarly, serum NO levels were also high in TT homozygote of G274T exon 16.This suggested that iNOS expersion might be increased in the mutated homozygotes of the tested SNPs, leading to increased NO levels in serum. In G274T exon16 polymorphism, homozygote for T allele showed highest serum TNF–α level in comparison to GG or GT genotypes. T allele was the one which showed statistically significant association with PE (p=0.001, OR=2.50, 1.40<OR<4.4).TNF-alpha is an important cytokine for induction of NOS2A gene. Thus, it might be that there is relationship between increased TNF- α levels and NOS2A polymorphism leading to its altered expression in preeclamptic patients. For a better understanding of how the different SNPs of iNOS affect the expression of the gene, more extensive studies are required.

In conclusion, we provide evidence of an association between TNF-alpha and iNOS in preeclampsia and for the possible role of G300A Exon8 and G274T Exon16 in the pathogenesis of this disease.

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