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Association of IFN-γ, IL-4 and Interleukin-10 Gene Polymorphisms with Preeclampsia

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ABSTRACT

Background: In view of significant effect of cytokine-polymorphisms on inflammation and immune regulation, and Inflammatory cytokines causing Preeclampsia (PE), it becomes mandatory to examine the role of cytokine-SNPs in all pathological mechanisms responsible for PE. In this review, we describe association between polymorphisms in IFN-γ (+874 A/T; rs2430561), IL-4, IL-10 (-819), IL-10 (-1082), IL-10 (-592) genes and preeclampsia and healthy controls in North Coastal Andhra Pradesh of India.
Methods: 200 samples (100 preeclamptic and 100 normal pregnant women as control group) were sequenced using allele-specific oligonucleotides-polymerase chain reaction and PCR-RFLP. Data was analyzed using chi-square and Fisher's exact tests.
Results: IL - 4 (C590T) C/T genotype (odds ratio 2.452, 95% confidence interval (1.299-4.626); P=.002) and genotype CC of IL-10 (C819T) (odds ratio 0.4361, 95% confidence interval (0.2377-0.8001); P=.003) showed a significantly higher frequency among the preeclamptic group than the control group.
Conclusion: Genotype CT of IL-4 (C590T) and genotype CC of IL-10 (C819T) have a significant role in the development of preeclampsia; differing in `genetic predisposition/pathophysiology.

Keywords: IFN-γ, IL-4, IL-10, Interleukin-10, Gene Polymorphism, Preeclampsia

INTRODUCTION

Preeclampsia (PE) is a multiorgan dysfunction disorder, affecting 2% to 8% of mothers in the second half of pregnancy, significantly contributing to fetal and neonatal morbidity and mortality, especially in developing countries. [(1)] Various factors, including genetics, environment, and immunology, lead to endothelial dysfunction and organic failure[(2)]. Some women, due to genetic predisposition, are at a higher risk of developing PE. Familial PE is associated with a more severe phenotype, and about 30% to 35% of heritability is attributed to the maternal genotype [(3-4)]. Genome-wide association studies (GWAS) have identified specific SNPs involved in cytokine-mediated endothelial damage pathogenic processes associated with PE [(5)]. Immunological factors, including excessive innate immunity and altered cytokine profiles such as overexpression of Th1 immunity tumor necrosis factor-alpha (TNF-α) and interferon-gamma cytokine (IFN-γ) and low interleukin 4 (IL-4), interleukin 10 (IL-10) and transforming growth factor beta receptor 1 (TGF-β1) and altered interleukin 2 (IL-2) , IL-2/IL-10 and

TNF-α/IL-10 ratios, interleukin 17 (IL-17) and interleukin 22 (IL-22), play a role in PE predisposition [6,7]. Suggested by a large study that individual cytokines may not reliably identify PE, a panel of proinflammatory cytokines for diagnosing PE should be identified[8]. Despite reported associations between PE and cytokine gene SNPs, the relationship remains unclear due to different selection criteria [9 -11]. This review aims to identify the association between specific SNPs in genes like IFN-γ, IL-4, and IL-10 with PE in North Coastal Andhra Pradesh of India.

MATERIALS AND METHODS

100 Preeclampsia Women and 100 Healthy Pregnant Women as controls belonging to North Coastal Andhra Pradesh, who attended the Out Patient Department (OPD) of Obstetrics and Gynecology Unit of King George Hospital and as well as admitted in the antenatal and postnatal wards of the hospital were enrolled based on the inclusion and exclusion criteria. (Supplementary Table 1). The predesigned questionnaire containing relevant information pertaining to demographic parameters and medical history was filled by the participants. The approval by the institutional ethical committee and the informed consent was taken.

Sample collection and analysis

DNA was isolated from a 3-mL whole blood sample of all the participants using the standard method of salting out. We studied IFN-γ (+874 A/T; rs2430561), IL-4 (rs2243250), IL-10 (-819) rs1800871, IL-10(-1082) rs1800896, IL-10 (-592) rs1800872 polymorphisms.

Polymorphism of IFN-γ (+874 A/T) in the Intronic region**, IL4 in -588C>T region** and **IL-10 (-819), IL-10 (-1082) and IL-10 (-592)** were screened by polymerase chain reaction (PCR)-based methods. Optimized PCR conditions for the studied cytokines and Protocols used for genotyping the selected molecular markers for cytokine gene polymorphisms have been provided in (Supplementary Table 2). Aliquots of the PCR products were analyzed on 2% agarose gel stained with ethidium bromide to verify the proper amplification of the fragments.

STATISTICAL ANALYSIS:

Genotype distribution in the control and case groups were compared with values predicted by Hardy-Weinberg equilibrium analyses were performed using Fisher's exact and chi square tests. The results were considered to be significant when the *p*-value was less than 0.05. Odd ratios (OR) and their 95% confidence intervals were used to measure the strength of association between IFN-γ (+874 A/T; rs2430561), IL-4, IL-10 (-819), IL-10 (-1082), IL-10 (-592) gene polymorphism and preeclampsia.

RESULTS

In this study a total of 100 women with preeclampsia and 100 normal controls were analyzed for carrying IFN-γ (+874 A/T), IL-4 (C590T), IL-10 (C819T), IL-10 (1082G/A), IL-10 (592C/A) polymorphisms. The allelic frequency and genotype frequencies of the studied polymorphisms are summarized in Table 1 & 2. As shown in the table 1, The chi-square p-value of IFN-γ (+874 A/T), IL-4 (C590T), IL-10 (C819T), IL-10 (1082G/A), IL-10 (592C/A) polymorphisms show no association with preeclampsia. Table 2 shows the odds ratio p value of genotype CT of IL-4 (C590T) and genotype CC of IL-10 (C819T) showed a significantly higher frequency among the preeclamptic group than the control group (odds ratio, 2.452, 95% confidence interval, (1.299-4.626); *P* = .002; odds ratio 0.4361, 95% confidence interval, (0.2377-0.8001); *P* = .003 respectively).

Table 1: Allelic frequencies of INF-γ (874A/T), IL-4 (C590T), IL-10 (C819T), IL-10 (1082G/A), IL-10 (592C/A) in Preeclampsia Cases and Controls show no association with preeclampsia.

Alleles	Preeclampsia Cases		Controls		p value
	n=100	Frequency (%)	n =100	Frequency (%)	
INF-γ (874A/T)					
T	94	(47%)	87	(43.5%)	0.42^{NS}
A	106	(53%)	113	(56.5%)	
IL-4 (C590T)					
C	154	77%	168	84%	0.202 ^{NS}
T	46	23 %	32	16 %	
IL-10 (C819T)					
C	112	56 %	134	67%	0.150^{NS}
T	88	44 %	66	33 %	
IL-10 (1082G/A)					
G	80	(40%)	76	(38%)	0.340^{NS}
A	120	(60%)	124	(62%)	
IL-10 (592C/A)					
C	126	(63%)	134	(67%)	0.401^{NS}
A	74	(37%)	66	(33%)	

n = number of alleles, p value = probability value of the statistical test, S= significant, NS= not significant

Table 2: Genotypic frequencies of INF-γ (874A/T), IL-4 (C590T), IL-10 (C819T), IL-10 (1082G/A), IL-10 (592C/A) in Preeclampsia Cases and Controls. The odds ratio P values of genotypes CT of IL-4 (C590T) and genotype CC of IL-10 (C819T) were statistically significant.

Genotype	Preeclampsia Cases n=100 (%)	Controls n=100(%)	Total n=200 (%)	Odds Ratio	95% CI	p value
INF-γ (874A/T)						
TT	23 (23%)	18 (18%)	41(20.5%)	1.46	(0.7336-2.907)	0.14 ^{NS}
TA	48 (48%)	51 (51%)	99(49.5%)	0.8869	(0.5093,1.544)	0.33 ^{NS}
AA	29 (29%)	31 (31%)	60(30%)	0.9091	0.4964-1.665	0.37 ^{NS}
IL-4 (C590T)						
CC	58 (58%)	74 (74%)	132 (66%)	0.4852	(0.266-0.8821)	0.008^{NS}
CT	38 (38%)	20 (20%)	58 (29%)	2.452	(1.299-4.626)	0.002^{S}
TT	04 (4%)	06 (6%)	10 (5%)	0.6528	0.1785-2.387)	0.258 ^{NS}
IL-10 (C819T)						
CC	24 (24%)	42 (42%)	66 (33%)	0.4361	(0.2377-0.8001)	0.003^{S}
CT	64 (64%)	50 (50%)	114 (57%)	1.778	(1.009-3.131)	0.022^{NS}
TT	12 (12%)	08 (08%)	20 (10%)	1.568	(0.6119-4.019)	0.172 ^{NS}
IL-10 (1082G/A)						
GG	19 (19%)	22 (22%)	41 (20.5%)	0.8316	(0.418-1.655)	0.29 ^{NS}
GA	42 (42%)	32 (32%)	74 (37.0%)	1.539	(0.8632-2.743)	0.071 ^{NS}
AA	39 (39%)	46 (46%)	85 (42.5%)	0.7505	(0.4278-1.317)	0.159 ^{NS}
IL-10 (592C/A)						
CC	40 (40%)	42 (42%)	82 (41%)	0.9206	(0.524-1.618)	0.38 ^{NS}
CA	46 (46%)	50 (50%)	96 (48%)	0.8519	(0.4889 -1.484)	0.28 ^{NS}
AA	14 (14%)	08 (8%)	22 (11%)	1.872	(0.7483-4.684)	0.08 ^{NS}

n = number of alleles, p value = probability value of the statistical test, S= significant, NS= not significant

DISCUSSION

Heterogeneous nature of preeclampsia can obscure eventual associations between gene polymorphisms and the disease and lead to discordant results in different studies involving the same disorder [12-13]. The genes we studied, IFN-γ (+874 A/T; rs2430561), IL-4 (rs2243250), IL-10 (-1082) rs1800896, IL-10 (-592) rs1800872, are related to known pathophysiological features in PE. However, it is likely that no single PE gene exists; because; clusters of the PE genes will exist in different populations. The expression of IFN- γ along with the other cytokines (IL- 4, IL-6, IL-8, TNF-α) have a key role in the activation of immune effector cells at the feto-maternal interface.

In the present study IFN-γ (+874 A/T) polymorphism shows comparatively higher frequency of TA heterozygote (48% in cases vs. 49.5% in controls) and normal AA homozygote (29% in cases vs. 31% in controls) and a lower frequency of mutated TT homozygote (23% in cases vs. 18% in controls), with a non- significant differences between the two study groups, in accord with studies which did not observe any significant difference between PE cases and controls for the distribution of IFN-γ (+874 A/T) polymorphism [14-15]. These conflicting findings could have resulted

from the heterogeneity in study designs, definition of phenotype, population diversity and sample size.

In contrast, de Lima et al., 2009 study showed higher frequency of IFN-γ + 874 A in eclamptic women compared to controls [16]. These results could have occurred by chance, since authors did not detect a corresponding expression in genotype frequency. Melina Pinheiro et al., 2015 study suggested IFN-γ +874 T/T genotype is associated with severe PE in the studied population (only severe PE cases were investigated) [11].

In IL – 4(C590T) variant, CC genotypic frequency was higher in controls 74 (74%) than in PE cases 58 (58%). The genotypic frequencies of CT and TT were 38 (38%) and 04 (4%) in PE cases whereas in controls the genotype frequencies of CT and TT were 20 (20%) and 06 (6%) respectively. The total frequencies of CC, CT and TT were 132(66%), 58 (29%) and 10(5%) respectively. The odds ratio p value of genotype CT was statistically significant with IL - 4 (C590T) variant. Previous study found similar results of marked trend for an association between IL-4 590 (C/T) SNP and PE with a prevalence of TT homozygous women in the study group [17].

Besides, Rajakumar et al., 2011 used microarray analysis for the gene expression of IL-4 , observed reduced gene expression of IL-4 in severe preeclampsia; reason can be diagnostic criteria which shows impact on the severity of preeclampsia affecting outcome [17- 18].

In IL - 10 (C819T) variant, the CC genotypic frequency was higher in controls 42 (42%) than in PE cases 24 (24%). The genotypic frequencies of CT 64 (64%) and TT 12(12%) in PE cases were higher when compared with CT 50 (50%) and TT 8(8%) in controls. The total frequencies of CC, CT and TT were 66 (33%), 114 (57%) and 20(10%) respectively. The odds ratio P value of genotype CC was statistically significant whereas the genotypes CT and TT were insignificant with IL - 10 (C819T) variant. Our results are in corroboration with the study of de Lima et al., 2009 who did not find any significant association between the IL-10 -1082A/G and -819T/C polymorphisms and the development of preeclampsia. [16] Sowmya et al. a, b, 2014 showed contradictory results, suggested that IL-10 -819T/C and -592A/C gene polymorphism could contribute to the risk of preeclampsia [19].

In IL - 10 (G1082A) variant, the genotypic frequencies of GG, GA and AA were 19 (19%), 42 (42%) and 39 (39%) in PE cases and in controls the genotypic frequencies of GG, GA and AA were 22 (22%), 32 (32%) and 46 (46%) respectively. The total frequencies of GG, GA and AA were 41(20.5%), 74(37.0%) and 85(42.5%) respectively. The odds ratio P value of the variant IL - 10 (1082G/A) was statistically insignificant, showed no significant association between IL-10 and PE. Previous studies have assessed that IL-10 -1082A/G polymorphism was associated with the risk of preeclampsia [9;14]. However, Stonek et al., 2008 reported lack of any association between the IL-10 gene polymorphism and risk of preeclampsia similar to the present study [10].

In IL - 10 (C592A) variant, the genotypic frequencies of CC,CA and AA were 40 (40%),46 (46%) and 14 (14%) in PE cases and in controls the genotypic frequencies of CC, CA and AA were 42 (42%),5 0(50%) and 08(8%) respectively.

The total frequencies of CC, CA and AA were 82 (41%), 96(48%) and 22 (11%) respectively. The odds ratio P value of the variant IL - 10 (592C/A) was statistically insignificant, no significant association was observed in the dominant, recessive or co-dominant model and probably more studies are needed to investigate the way this polymorphism affects the predisposition to preeclampsia .

A recent meta-analysis suggests that IL-10 -1082 G/A, -819 C/T and -592 C/A polymorphisms are unlikely to be associated with pre-eclampsia [10; 16].

LIMITATION

This study was conducted in a genetically homogeneous population, limiting the generalizability of results to other populations and ethnic groups. Additionally, the study's small sample size, though consistent with earlier research, is a limitation [20].

CONCLUSION

Ethnic differences are crucial in disease gene association studies. Future studies specific to the Indian population are essential. Given the involvement of multiple genes, a strategy involving multilocus or haplotype analysis, rather than single locus studies, is recommended for more accurate results in exploring the link between genetic susceptibility and preeclampsia.

Declaration by Authors

Ethical Approval: Approved

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