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A association study on inflammatory cytokine polymorphism (TNF alpha) with pre term birth.

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Abstract
Preterm is defined as babies born alive before 37 weeks of pregnancy are completed. An estimated 15 million babies are born too early every year. That is more than 1 in 10 babies. Approximately 1 million children die each year due to complications of preterm birth (1)*. In present research work we selected and studied distribution pattern of inflammatory biomarkers TNF alpha gene variants in the preterm birth (case) of central India. Detection of TNF-α promoter polymorphism has been done via PCR-RFLP. The pattern of genotype and allele distribution in PTB and control group suggested lack of association of TNF-α (rs1800629) in PTB susceptibility. Genotype distribution, allele frequency and carriage rate suggest TNF –α polymorphism is not associated with pre term birth.*

Keywords: *Preterm birth, inflammatory cytokines, TNF-α*

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I. Introduction

Preterm is defined as babies born alive before 37 weeks of pregnancy are completed. An estimated 15 million babies are born too early every year. That is more than 1 in 10 babies. Approximately 1 million children die each year due to complications of preterm birth. These babies are known as preemies or premies. Symptoms of preterm labor include uterine contractions which occur more often than every ten minutes or the leaking of fluid from the vagina. Premature infants are at greater risk for cerebral palsy, delays in development, hearing problems and problems. The research in last two decades has accumulated evidence to strong association of gene in preterm birth. Mutations in these candidate genes lead to genetic polymorphism (variation in DNA sequence) among random mating individuals, groups, or population due to the effects of environment. These mutations may be either a single nucleotide polymorphism (SNP) or a variable number of tandem repeats (VNTR) of a short repetitive DNA sequence that may influence the rate of gene transcription, the stability of the messenger RNA (mRNA), or the quantity and activity of resulting protein .Thus, the susceptibility or severity of preterm birth will be influence by possession of specific alleles of polymorphic genes. Cytokines are a group of soluble proteins secreted by the cells that can increase or decrease the inflammation response. Cytokines maintain homoeostasis during pregnancy and play crucial roles in regulating placentation (Kayaalt Z, et al 2011)A delicate balance between proinflammatory and anti-inflammatory cytokines regulates the inflammatory response during pregnancy (Yılmaz Y 2012). Inflammatory cytokines are previously found to be elevated during the condition of pre term birth therefore our major interest was to investigate the genetic polymorphism and allelic variation of inflammatory genes. *TNF-α* is located on chromosome 6 (6p21.3). It is a pro-inflammatory cytokine, which promotes the production of collagen-degrading matrix metalloproteinases, and suppresses biosynthesis of tissue inhibitors of metalloproteinases. (So T et al., 1992, . Anum EA et al., 2009). The metalloproteinases act on foetal membrane collagen resulting in loss of tensile strength. It also impairs the progesterone stimulating receptor B thus blocking the progesterone release.

II. Material and Methods

Preterm birth was considered the delivery of an infant between 20 and 37 weeks of gestation (Martin et al., 2005). The study population consisted of case (n=150) were neonates from pregnancies complicated by spontaneous PTB and controls (n=180) were neonates delivered at term (≥ 37 weeks of gestation) but not after 42 weeks of GA. Height and Weight were measured in light clothes and without shoes in standing position as

per standard guidelines. Body Mass Index (BMI) was calculated as weight in kilograms divided by height in meters squared.
DNA isolation has been done as per suggested protocol of miller et al 1987 with few modifications. Genomic DNA was extracted from whole blood by the modification of salting out procedure and isolated DNA sample was subjected to electrophoresis and UV spectrophotometry for quantification.
Detection of TNF-α promoter polymorphism via PCR-RFLP

Primers
The oligonucleotides (primers) used were the sequences flanking this region described before . They are as follows:
TNF -α forward primer - 5' AGG CAA TAG GTT TTG AGG GCC AT3'
TNF -α reverse primer – 5'TTG GGG ACA CAC AAG CAT CAA GG3'

PCR Mix
25 µl of each PCR reaction mixture contained 2-5 µl template DNA (final concentration 100-200 ng/ µl), 2.5 µl of 10X Taq polymerase buffer (10 mM Tris HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl2, 0.01% gelatin, 0.005% Tween-20, 0.005% NP-40; final concentration 1X; Genetix Biotech Asia Pvt. Ltd.,India), 1 µl of 10 mM dNTPs (Banglore Genei, Bangalore, India), 1 µl of 10 pm/µl of forward and reverse primers specific for IL-1Ra gene, 0.3 µl of 5U/ µl of Taq DNA polymerase (final concentration 1.5U; Banglore Genei, Bangalore, India) and sterile water to set up the volume of reaction mixture to 25 µl.

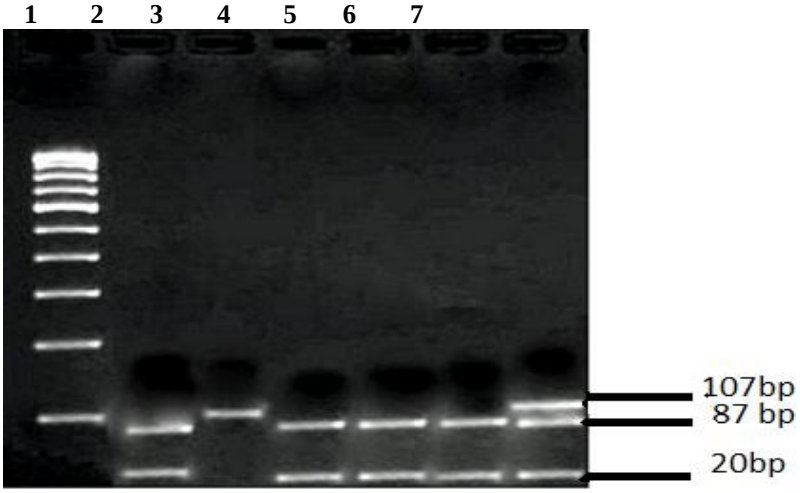

Figure: 10. Representative gel picture of TNF-α -308 G<A polymorphism

Lane 1: DNA marker
Lane 2, 4, 5, 6: GG (87bp and 20 bp)
Lane 3: Undigested
Lane 7: GA (107bp, 87bp and 20bp)

Association of TNF-α -308 genotypes, alleles and their carriage rates with susceptibility to disease in PTB compared to controls using Fisher exact test.

TNF-A GENOTYPES	CASE N= 150		CONTROL N=180		P VALUE	ODDS RATIO AND CI
	N	%	N	%		
GG	107	71.3	142	78.9	0.1241	0.6659 (0.4025 to 1.102)
GA	39	26.0	35	19.4	0.1851	1.456 (0.8661 to 2.4460)
AA	04	02.7	03	1.7	0.7060	1.616 (0.3559 to 7.341)
ALLELES						
G	253	84.3	319	88.6	0.1093	0.6919 (0.4410 to 1.085)
A	47	15.7	41	11.4		

CARRIGE RATE						
G	146	97.3	177	98.3	0.3491	0.7390 (0.3998 to 1.366)
A	43	22.7	38	21.1		1.353 (0.7321 to 2.501)

N – Number of individuals carrying particular genotype in a study

% - Genotype frequency, allele frequency & carriage rate in percentage

***-** Significant values

χ^2 (*P* Value) - indicates χ^2 *P* Value when control is compared to PTB

III. Results

TNF-α is an important regulatory molecule during pregnancy, which mediates an inflammatory response and is also involved in labor activities such as membranes repture and uterine contractions. In this investigation Genotype distribution of control group 'GG' (78.9%) showed increase in 'GG' genotype as compared to Preterm birth group (71.3%) but considerably not significant. An odds ratio of 0.6659 in PTB group for 'GG' genotype showed a protective effect of this less common in our PTB group of vindhya region. Heterozygous 'GA' genotype was present in lower frequency in control (19.4%) as compared to PTB group (26.0%) but significantly different. An odds ratio of 1.456 in PTB group for 'GA' genotype indicated no involvement in Preterm birth. Similarly, the homozygous genotype 'AA' was found non significantly 2.7% in PTB group and 1.7% is distributed in control group. An odds ratio of 1.616 for 'AA' genotype was constant with slight or no consequence with PTB susceptibility. Over all allele distribution was non significant but 'G' allele was found to be low frequency in PTB group as compared to control group (84.3 vs 88.6) whereas allele 'A' was present in significantly high frequency in the PTB group as compare to control group (15.7 vs 11.4) but the difference was non significant (χ^2 = 1.876, P=0.1891). Genotype distribution, allele frequency and carriage rate suggest TNF –α polymorphism is not associated with pre term birth.

IV. Discussion

Increased level of TNF-α was linked with various reproductive diseases such as frequent spontaneous abortions, pre-eclampsia, infections or endometriosis57. Elevated levels of TNF-α can change the delicate equilibrium between the anti-inflammatory and pro-inflammatory cytokines and thus induce PTB. Etiology of PTB is still unknown because PTB is a complex syndrome with a variety of causes, involving a complex interaction between genetic and environmental factors. Clinical infection, a low progesterone level, multiple pregnancy, a short cervix and placental aberrations are regarded as important risk factors (Pařízek A, at al 2014, Lutenbacher Met al 2013). Morra et al. demonstrated by genotyping of 410 Brazilian ethnically matched women that the combination of TNF-α maternal gene polymorphisms might contribute to the susceptibility to sPTB and may be regarded as possible genetic markers of the risk of sPTB. Our finding shows lack of any association of TNF alpha promoter polymorphism with pre term birth in central Indian population but Significant association of *TNF-α*(-308G/A) polymorphism has been reported with PTB (Pu J et al., 2009, Jones NM et al., 2010, Liang M et al., 2010, Yılmaz Y et al., 2012)

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