

Genetic evaluation of *tumor necrosis factor-alpha* gene polymorphisms with pre and post term births

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ABSTRACT

Tumor necrosis factor alpha is known as multifunctional cytokine component of apoptosis and cell survival. Preterm delivery is a confused prenatal unfavorable occasion. This examination work was planned to associate relationship of G308A and -238 polymorphisms in Tumor necrosis factor alpha. We collected blood samples of 70 patients out of which 50 ladies gave untimely births and 20 ladies gave typical births. DNA extraction technique was applied. Primers were designed, amplified by PCR and RFLP were performed to identify the TNF- α -308 and -238 polymorphisms. The frequencies of TNF- α -308 genotype TNF GG, GA and AA were 37%, 10% and 3% with measurably estimation of 0.07039 ($P < 0.05$) as indicated by HWE for the preterm and postterm conveyances while for the typical one proportion of GG, GA and AA were of 5%, 10% and 5% with factually critical esteem is 1.000 ($p < 0.05$). Conclusion: If there should happen an occurrence of TNF- α -238 genotype of GG, GA and AA were 35%, 12% and 7% of those ladies who conveyed untimely births and in typical ones factually critical esteem was additionally more than 0.05. The significance level of 0.005872 of TNF- α -308 and 0.010089 in TNF- α -238 as indicated by Exact Fisher test are important. However, further study on large sample size would increase the risk of pre and post term births in Lahore.

Keywords: Polymorphisms, *Tumor necrosis factor-alpha*, Gene, PCR, Preterm birth

INTRODUCTION

Internationally a large number of newborn children are conceived without finishing their ordinary development period. The important issue in neonates is preterm birth (PTB) due to complications, high mortality and morbidity. Preterm birth rate is 12-13% in USA and 5-9% in other developed countries. In China, 6.8% babies had premature birth. Preterm birth rate increasing at high rate throughout the world. Major risk factors of preterm birth are the environmental factors and genetic susceptibility. Changes in TNF α are viewed as the real reason pregnancy end and preterm conveyance (Tornatore et al., 2014).

In the developing nations, the preterm birth is around 1 out of 10 and around 1 million children kick the bucket because of intricacies and the individuals who survive, end up noticeably incapacitate sound-related and outwardly. For the most part developed babies kick the bucket younger than 5 days (Blencowe et al., 2012).

A couple of examinations have been

directed to review the connection between this SNP and unconstrained preterm birth. Regardless, data revealed that the relationship of SNP -308G/A TNF- α and unconstrained preterm birth TNF- α level differ essentially among race and ethnic social occasions. Accordingly, examinations of the piece of this SNP on unconstrained preterm birth in other race and ethnic social events are requiring. The objective of this present examination was to choose the connection between SNP -308G/A TNF- α and unconstrained preterm birth among the Acehnese ethnic social occasion (Amir, Iryani, & Isrona, 2016).

Tumor Necrosis factor alpha (TNF- α) is a polypeptide genius incendiary cytokine show in uterus of pregnant ladies that reasons for some variations from the norm amid birth. TNF α is a key master provocative cytokine. Obviously cytokines, including TNF- α , are vital amid pregnancy and preterm work (Chen et al., 1991). The TNF- α was an essential factor related with preterm birth particularly with amniotic liquid fixation was related with preterm birth among African-

American race (Anum, Springel, Shriver, & Strauss, 2009).

TNF alpha is arranged on chromosome 6. A single nucleotide polymorphism from Guanine (G) in standard allele of TNF to Adenine (A) in the TNF variant at position 308, result in two allelic structures, in which the closeness of adenine portrays the less common variety (Adams, 2004).

Tumor putrefaction factor alpha is an intense cytokine that has an extensive variety of star incendiary exercises, its progress from G to A at position 308 builds its creation amid pregnancy that reasons for unconstrained fetus removal and preterm birth (Romero-Corral et al., 2006).

Mothers, who don't take angle amid pregnancy, additionally have high danger of preterm conveyance, since fish or fish-oil gives security against preterm conveyance and low birth weight in ladies with zero or low admission of fish. Pregnant ladies of Pakistan have abnormal amounts of shifting dental ailment. Press inadequacy sickness expanded the danger of preterm conveyance by two times (Volianitis et al., 2012).

Postterm pregnancy is characterized as pregnancy that keeps going longer than 42 weeks, two weeks past the typical 40 weeks growth period. Postterm pregnancy is connected with both fetal and maternal wellbeing complexities. For most of the times, post term pregnancy involves wrong estimation of feminine cycle date.

TNF- α is a basic managerial molecule in the midst of pregnancy and its age is coordinated at transcriptional as well as post-transcriptional levels. A couple of single nucleotide polymorphisms (SNPs) has been recognized inside TNF- α quality, in a SNP, homozygote AA and heterozygote GA are connected with extended TNF- α quality verbalization appeared differently in relation to homozygous GG (Drews-Piasecka et al., 2014).

TNF- α also is a key factor in cerebrum harm. In spite of the way that the revelation of TNF- α in the midst of common emotional wellness is continually growing in the midst of the late fetal period, over production of TNF- α by microglial cells was seen to be connected with the pathogenesis of neonatal leukomalacia (Sriram & O'Callaghan, 2007). Purpose of this study is to demonstrate the linkage connection between TNF alpha and preterm birth, this research work explains the relationship of TNF alpha 308 with preterm birth due to inconvenient delight of membrane.

MATERIALS AND METHODS

Sample collection

Hospitalized patients for deliveries with pre

and post term family history has identified and registered for this study after informed consent. Females (n=50) with history of normal and pre/post births have collected. Blood samples 3-5 mL from 50 patients has collected from Services Hospital Lahore, Lady Acheson hospital and Jinnah Hospital Lahore. Samples were taken by those patients who had pre and post term birth in their family history. These samples shifted into vacutainer containing EDTA as an anticoagulant and stored at -20°C in the lab for further processing.

DNA extraction

The whole blood samples used for DNA extraction used standard organic or Inorganic method with some modifications (Sambrook & Russel, 2000). DNA quantified using Nano Drop and agarose gel electrophoresis.

Following method used for DNA extraction of blood samples.

Took 200 μ L of blood sample, add 1mL buffer solution, Vortex mixed for 5 minutes. Centrifuged for 15 minutes at 4°C with 13000 rpm, discard the supernatants. Break down the pellet formed at the end. 20 μ L proteinase K (PK) and 80 μ L of SDS (Sodium dodecyl sulphate) added with 250 μ L of buffer A1. Vortex mixing for 5 minutes. Samples put in water bath for overnight at 58°C. Added 200 μ L PCI (Phenol chloroform isoamyl alcohol). Centrifuged for 10 minutes at 4°C with 13000 rpm/min. Took aqueous solution (supernatants) and added Isopropanol 1:1 ratio. Centrifuged and used 200 μ L Ethanol in samples for washing purposes. Centrifuged for 10 minutes at 4°C with 13000 rpm, discarded ethanol and put the samples for dry. Added 50 μ L Low TE (Tris HCL EDTA) buffer and save it at -20°C.

Agrose gel electrophoresis

To find out the results of DNA bands whole samples run on Gel. Took 0.4g agarose added 50ml TAE, heated it until boiled, added 30 μ L of ethidium bromide in it, when it solidifies, placed it in gel electrophoresis apparatus at 110V for 25 minutes. After gel running process visualize the DNA quantifying bands under GEL DOC apparatus.

Primers used for amplification

The selected part of the *TNF alpha* gene amplified using PCR protocol. Primers were designed using Primer 3 Plus software. Then synthesized and optimized.

Table I: TNF alpha primers promoter region

Primer ID	Sequence (5'- 3')	GC (%)	Tm	Length (bp)	Product Size (bp)
TNFA-308-F	AGGCAATAGGTTT TGAGGGCCAT	48	64.6	23	107
TNFA-308-R	TCCTCCCTGCTC CGATTCCG	48	67.6	20	
TNFA-238-F	AGAAGACCCCC CTCGGAACC	65	64.5	20	152
TNFA-238-R	ATCTGGAGGAA GCGGTAGTG	55	57.8	20	

Amplification through PCR

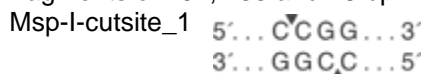
To get the PCR product used Touchdown PCR method. It is a strategy for expanding specificity of PCR responses. Touchdown PCR was utilized for amplification.

RFLP digestion

The product of *TNF- α* -308 i.e. 107bp was digested by *Nco*1 restriction enzyme according following the manufacturer's instructions. (*Nco*-I-cutsite_1)



For *TNF- α* (-238G/A), *Msp*I digested PCR products were of two types- a complete *Msp*I cut yields 152 bp fragment and two fragments of 133 and 19 bp for the wild-type allele and a partial cut which represents heterozygous, produced three fragments of 152, 133 and 28 bp.

**RESULTS**

Our analysis included total 70 blood samples of pregnant women having family history of pre and post maturity. Following results found after analysis:

Amplification yielded 152bp PCR product of -308G/A fragment visualized by using 2% agarose gel run along with DNA ladder of 50bp.



Fig.1: Gel Electrophoretic analysis of PCR samples; 107bp band representing PCR amplicon of *TNF- α* -308 selected region.

Restriction fragment length polymorphism (rflp)

Enzymatic digestion was done by using *Nco*1 and *MSP*1 restriction enzymes then PCR-RFLP products were visualized on 3.5% agarose gel. There were three types of bands observed, homozygous *TNF- α* (-308G/G), which results in two fragments of 87 and 20 bp and heterozygous band of *TNF- α* (-308G/A).

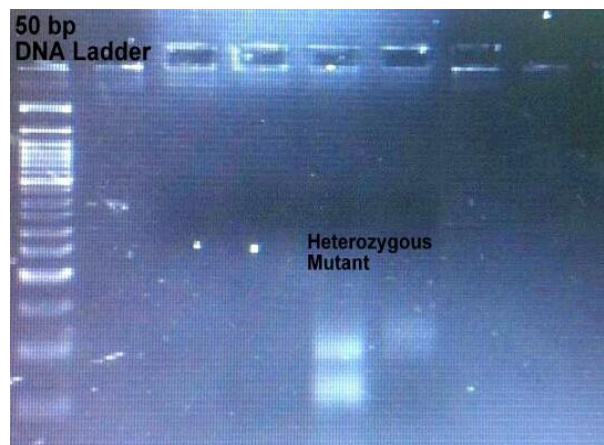


Fig. 2: Agarose gel, displaying *TNF- α* -308 heterozygous mutation with single visible bands of 107 bp.

Data analysis

The alleles in each marker were accessed after RFLP analysis. The allele frequencies of both positions were calculated for Hardy-Weinberg principle. The control and affected groups were categorized based on the allele type. Association of -238 *TNF-alpha* was calculated using Fisher exact test is 0.010089 statistical values. The result is significant at $p < 0.05$.

Table II: Represents the Allele frequency of -238 *TNF-alpha*

Category	Allele G	Allele A	Significance Level
Controls	43	7	P=0.010089
Diseased	11	9	
Total	54	16	

The values for -308 *TNF-alpha* and its association with preterm and postterm births were accessed using Fisher exact test. The statistical values (0.005872) depicts that it is significant at $p < 0.05$.

Table III: Represents the Allele frequency of -308 TNF-alpha

Category	Allele G	Allele A	Significance Level
Controls	42	8	P=0.005872
Diseased	10	10	
Total	52	18	

Genotype frequencies of preterm births and conventional were not imperative ($p < 0.05$). The frequencies of TNF-alpha -308 genotype TNF GG, GA and AA were 37%, 10% and 3% with quantifiably immense estimation of 0.0703 ($P < 0.05$) according to HWE for the preterm and postterm movements while for the run of the millions extent of GG, GA and AA were of 5%, 10% and 5% with truthfully basic regard is 1.000 ($p < 0.05$).

In the event that there ought to be an event of TNF-alpha -238 genotype of GG, GA and AA were 35%, 12% and 7% of those women who passed on less than ideal births and in customary ones authentically immense regard was moreover more than 0.05. The allele frequencies of PTB were 0.005872 if there ought to emerge an event of TNF-alpha -308 and 0.010089 in TNF-alpha -238 according to Exact Fisher test. All these significant results depict that TNF alpha genetically associated with pre and post term births, by using TNF inhibitors rate of pre maturity can be reduced.

DISCUSSION

Preterm birth likely incorporates different common and genetic peril factors. Various women passing on preterm have no known risk factors. In this way, developing our understanding into PTB danger and causality is essential to the disclosure and utilization of practical intervention and treatment.

The examinations reviewed here are the underlying stages in dissecting the connection between innate assortment and PTB danger. In any case, the cases are close to nothing and are from time to time solace of high danger masses at implied facilities. The definition and estimation of the examination comes about are changed and were all things considered not planned to take a gander at the gene– quality and gene– condition associations related with PTB danger. Expansive replication close by information on racial/ethnic social occasions and subgroup examination is required before the impact of individual polymorphisms on PTB can be assessed; however results to date are intriguing.

Most examinations depicted here found that maternal or fetal carriage of essential polymorphisms in the aggravation pathway were connected with PTB shot. Generally, polymorphisms that development the enormity or traverse of the red hot responses were connected with extended PTB, and polymorphisms that reduction the response were connected with lessened PTB. This relationship concurs with epidemiologic verification suggesting that regular factors that extension exacerbation in like manner increase PTB shot. The TNF (308A) allele was broke down most as frequently as could be expected under the circumstances, and in numerous examinations it was connected with extended PTB. Two examinations propose biologic instruments for this association.

Hernandez-Guerrero demonstrated that in vitro amniochorion tissue with the TNF (308A) allele responded in like manner to lipopolysaccharide (surface lipopolysaccharide on Gram-negative microorganisms, which triggers a safe reaction) as the genuine allele, beside at higher lipopolysaccharide estimations.

The tissue with the TNF (308A) allele made through and through more raised measures of TNF– than the tissue with the huge allele. This proposes tissues imparting TNF (308A) may hyper respond to a uterine infection by releasing a great deal of TNF.

Simhan found that maternal TNF (308A) allele in term pregnancies extended the risk of chorioamnionitis by 3.3– overlay (95% CI; 1.3– 7.1), 38 prescribing that the TNF (308A) allele may in like manner be locked in with pollution mediated preterm birth.

Starting late Macones found that the TNF (308A) allele was connected with extended preterm delivery (OR 2.7, 95% CI 1.7– 4.5), 113 and that the alliance was essentially more grounded inside seeing BV (OR 6.1, 95% CI 1.9 – 21.0). In a running with review, Romero accomplices guessed that BV may slant women with a particular blend of polymorphisms to preterm birth and that various polymorphisms may be locked in with propelling a hyper safe or hypo insusceptible response. Generally speaking, the examinations reviewed here are unsurprising with this model and there may be various additional multi factorial gene– quality and gene– condition affiliations that convey a hyper immune or hypo safe responses that could provoke PTB. TNF α quality creation is mostly controlled at transcriptional level. The position –308 in TNF α quality may have a run of the million guanine (G)

allele or have a variety adenine (A) allele.

It has been shown that G308A advance in the promoter zone of TNF- α quality results in TNF- α AA or GA genotypes that may start bigger measures of TNF- α quality translation and order and cause sickness feebleness in the human subjects. In show look at we found high rate of homozygous wild sort when appeared differently in relation to heterozygous and homozygous mutant. In perspective of the mix of the above affirmation, a couple of pros have assessed that preterm work may be connected with inherited changes in TNF- α quality at a couple of positions, for instance, -308. A number of studies recommend that this genotype variety may impact the likelihood of PTD, however some others reject this alliance.

In 2005 Fiscella given that common factor unmistakably add to preterm delivery. A significant part of these components generally impact to racial disparity in preterm labor. In this manner, actuating innate sense of duty regarding racial contrast in preterm birth by trying to control for environmental segments measured at a singular point in time is flawed. There is rising evidence of value condition correspondences for preterm birth, some of which disproportionately impact African Americans. There is also verification of racial differences in the inescapability of polymorphisms possibly related to PTD.

If TNF (308A) allele is found to progress preterm birth by completed emanation of TNF-, high-danger women could be treated with anti-TNF-drugs. There is no affirmation if these drugs are making life unsafe or, then again teratogenic, yet thorough examination would be principal before they could be used for pregnant ladies.

This present examination demonstrates the linkage connection between TNF alpha and pre term birth, this work research an enormous relationship Of TNF alpha -308 with preterm birth due to inconvenient delight of membrane. PTB isn't a result of regular factor, it may be a direct result of multi factorial heritage. Extended rate of preterm delivery in women who had PTD, high probability of PTD in women with a previous history of PTD and examinations of pregnancy brings about twins suggest that genetic parts may be basic determinants of PTD.

Extended centralization of expert searing cytokines has been found in amniotic fluid, maternal and fetal blood of incalculable with PTD. Tumor defilement factor- α (TNF- α) is one of these a

specialist red hot cytokine that can trigger a course of events and provoke PTD. Enunciation of cytokines, for instance, TNF- α is furthermore under innate control.

It has been represented by Loiuise in 2006 that a genetic variety single nucleotide polymorphism (SNP) in the promoter region of TNF- α quality at -308 (a G/A change realizing TNF- α amino destructive (AA) or genotyping tests (GA) genotypes) may be connected with extended incitation of the TNF- α quality differentiated and the GG genotype.

Various distinctive observations suggest that women may have an inherited nature for pre and post term movement. A couple of examinations declared a connection between different levels of TNF alpha quality concentration in maternal blood or amniotic fluid and preterm movement.

A couple of examinations conjectured that preterm birth may be connected with inherited change in TNF alpha at a couple of positions, for instance, -308.

A lot of various examinations suggest that this genotype variety may impact the probability of PTB, yet some others expel this relationship at transcriptional level. The position -308 in TNF alpha quality may have a customary guanine or a variety adenine allele.

Different examinations prescribe that G308A change in the promoter region of TNF- α quality results in TNF- α AA or GA genotypes that may impel bigger measures of TNF- α quality interpretation and sanctioning and cause illness frailty in the human subjects.

Display consider demonstrated that notwithstanding higher repeat of GA change in TNF- α quality at -308 position in mothers and neonates with PTD, this connection was not quantifiably basic. Our revelations are enduring with a couple of past examinations, which did not find such connection. (Dizon-Townson, Major, & Ward, 1998) furthermore took a gander at the frequencies of TNF T1 or TNF T2 allele between women or children passed on preterm with the control social occasion or officially dispersed allele frequencies and point by point that the repeat of TNF- α promoter change isn't extended in either women or fetuses passed on preterm. In (Mishra et al., 2006), exhibited a meta-examination of accessible confirmation and mulled over that there is no quantifiably basic association between a SNP in the TNF- α quality PTD. Also, in vitro tests that used

-308 SNP and concentrates that used cells got from subjects with various TNF- α - 308 genotypes exhibited clashing results and brought up issues about the valuable criticalness of -308 SNP. The closeness of various SNPs in the TNF- α quality and moreover assortment in various qualities that can modify verbalization, elucidation and post-translational difference in TNF- α quality could be the feasible clarifications of this condition. (Weening et al., 2004) suggests that the mix of TNF- α , including interferon- γ and interleukin-6 maternal quality polymorphisms may add to shortcoming to PTD, not the TNF- α polymorphism alone.

Since immunological response is most probably the outcome of the collaboration between a couple of polymorphic qualities it isn't shock to find a critical connection between a lone polymorphism and an event, for instance, PTD.

CONCLUSION

The small sample size may be the limiting factor in concluding the role of Tumor Necrosis Alpha promoter area in -308 and -238 positions association with risk of pre and post term births in Lahore. However, this data suggest that both positions have impact on the PTB.

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