JOURNAL OF ADVANCES IN BIOMEDICAL STUDIES
http://www.jabsdirect.com

Research Article

Effect of the TNF α-308 polymorphism on birth outcomes among South African women

Poovendhree Reddy[1], Rajen N Naidoo[2], Anil Chuturgoon[3], Kareshma Asharam[2], Alisa Phulukdaree[3] and Shivona Gounden[3]

[1]Department of Community Health Studies, Durban University of Technology, Durban, South Africa.
[2]Discipline of Occupational and Environmental Health, School of Nursing and Public Health, University of KwaZulu-Natal, Durban, South Africa.
[3]Discipline of Medical Biochemistry, School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban, South Africa.

Correspondence Author:
Dr. Poovendhree Reddy
Department of Community Health Studies
Durban University of Technology
P.O Box 1334, Durban, 4000
Email:PoovieR@dut.ac.za

Received for publication: April 18, 2014; **Accepted:** April 22, 2014.

Abstract

The −308 G/A promoter polymorphism in the tumor necrosis factor-alpha (TNF- α) gene has been extensively studied as a potential biomarker for pregnancy outcomes, but results tend to be population specific. The aim of this study was to evaluate the association of the TNF α-308 polymorphism with preterm birth (PTB) and low birth weight (LBW) in a cohort of South African women enrolled in a prospective pregnancy study. The Mother and Child Environmental cohort (MACE) pilot study was done in Durban, KwaZulu-Natal during 2010-2011 with 100 pregnant women recruited. Demographic, exposure and prenatal clinical data was collected during the third trimester, maternal and infant hospital records at delivery were reviewed. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to determine the TNF-α -308 genotype. Plasma TNF-α concentration was measured using the human TNF-α Max Standard™ ELISA kit (Bio-legend). The polymorphic TNF-α GA+AA genotype was found among 35% of mothers. Mean birth weight was significantly lower among mothers with the TNF-α AG+AA genotype ($p<0.05$). Mothers who delivered LBW infants (<2500g) showed a significantly higher mean TNF- α level compared to mothers with normal birth weight deliveries. In addition, mothers with the TNF-α AG+AA genotype had a statistically significant drop in birth weight (β= -273.6; SE105.8; CI: -0.9,-1.35). While the TNF-α A allele was not associated with PTB, it was significantly associated with low birth weight in this study. Early identification of such immunological biomarkers may facilitate prevention of adverse pregnancy outcomes.

Keywords: TNF α-308, genetic polymorphism, birth cohort, birth outcomes

Introduction

Preterm birth (PTB) (<37 weeks gestation) and low birth weight (LBW) (<2500g) are associated with neonatal mortality and morbidity and have long term adverse consequences for health [1]. The morbidity associated with these birth outcomes often extends to later life, with increased physical, psychological and economic costs [2,3]. The burden of PTB and LBW is disproportionately concentrated in Africa; with the highest rate of PTB (11.9% of all births) and second highest rate (15%) of all LBW deliveries in the world [1,4]. These outcomes have thus been flagged as important indicators for Africa's progress towards the Millennium Development Goals. The causes of these events are multifactorial and etiology includes maternal health, genetic influences and environmental exposures.

Genes involved in the proinflammatory processes and the immune system may be involved in PTB. Tumor necrosis factor-alpha (TNF- α) is a potent cytokine which has a wide range of proinflammatory activities such as cell proliferation, differentiation and apoptosis [5,6].

Of the several polymorphisms identified in the gene, the −308 G/A promoter polymorphism has been extensively studied as a potential biomarker for pregnancy outcomes. A single nucleotide polymorphism (SNP) from a normal guanine (G) to a variant adenine (A) allele at position

http://www.jabsdirect.com
Copyright © 2014 JABS

308 in the promoter region has shown a twofold increase in transcription of the gene thus increasing both TNF- α concentrations and disease susceptibility [5,7]. As pregnancy develops, high TNF- α concentrations have been related to the development of preeclampsia, gestational diabetes, PTB, foetal growth restriction and other obstetric complications [6].

Early identification of such immunological biomarkers may facilitate prevention of adverse pregnancy outcomes. However, there has been conflicting results for the association of the TNF- α 308 gene with PTB and LBW and studies suggest that these may be population specific and the African environmental and socioeconomic context may influence this association. The Mother and Child Environmental Cohort (MACE) study, piloted in South Africa in 2010-2011, collected genetic, biochemical and clinical data from a sample of pregnant women. The aim of this study was to evaluate association of the TNF α-308 SNP with PTB and LBW in a cohort of women enrolled in a prospective pregnancy study.

MATERIALS AND METHODS

Selection of Ante-natal clinics and participants

The MACE (Mother and Child Environmental cohort) pilot study was done in Durban, KwaZulu-Natal during 2010-2011. Four ante-natal public sector clinics servicing communities of similar socio-economic profiles were selected. One hundred women were recruited in their 3rd trimester of pregnancy. Women were excluded if (1) they experienced the following complications: hypertension, diabetes, placenta previa, genital tract infection; (2) the first antenatal visit occurred >34 weeks and (3) this was a multiple pregnancy. Human immunodeficiency virus (HIV) status assessed during ante-natal testing was not an exclusion criterion. All pregnant females that met the inclusion criteria were recruited into the study. Participation was on a voluntary basis and only consenting women were enrolled. The purpose of the study was explained to all participating women. The Biomedical and Research Ethics Committee at UKZN approved the research protocol. Participants answered a questionnaire collecting demographic, clinical and exposure data. Prenatal clinical data was obtained from patient records. The women were followed up with regard to pregnancy outcomes. Maternal and infant hospital records at delivery were reviewed to obtain clinical data including pregnancy complications, and birth outcomes (gestational age and birth weight). The birth weight was measured in the delivery room by a trained nurse while the gestational age was assessed as time since the first day of the last menstrual period. Term delivery was defined as gestational age ≥ 37weeks while preterm delivery was defined as <37 weeks gestation.

TNF-α genotyping

Genomic DNA from mothers was extracted from blood collected in the third trimester according to standard protocols [8]. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to determine the TNF-α -308 genotype[9]. A PCR product was amplified using 20 *p*mol forward and reverse primer in a 25µl reaction containing 200 µM of each dNTP, 1.5mM MgCl2, 1X Green GoTaq Flexi buffer (Promega), 1U GoTaq DNA polymerase (Promega) and 100ng genomic DNA template. Following an initial denaturation step at 96°C for 5 minutes, amplification was carried out by 35 cycles of denaturation at 96°C for 30 seconds, annealing at 60°C for 30 seconds and extension at 72°C for 30 seconds. This was followed by a final extension at 72°C for 5 minutes. The restriction mix included 10 µl of the PCR product, 2 µl of 10X Buffer Tango™, 18 µl of nuclease-free water and 1.5 µl of 10 U/µl *Nco*I (Fermentas). Digestion of the PCR product produced the -308 G allele, which resulted in two fragments, 87 bp and 20 bp; and the –308 A allele which resulted in a single 107 bp fragment.

TNF-α ELISA

Plasma TNF-α concentration was measured using the human TNF-α Max Standard™ ELISA kit (Biolegend). A high-affinity microtitre plate was coated with 100 µl/well TNF-α capture antibody (18 hrs at 4°C). Plates were washed and treated with 200 µl assay diluent. Thereafter, 100 µl standards and samples were added and left to incubate (2 hours at room temperature). Plates were washed and 100 µl biotinylated anti-human TNF-α detection antibody (1 hour at room temperature) and avidin-horseradish peroxidase (30 minutes at room temperature) was added to each well. 100 µl of TMB substrate and 100 µl stop solution (1M H_2SO_4) was added to each well. Absorbance was measured at 450nm (Bio-Tek µQuant ELISA plate reader).

Data analysis

Statistical analysis was done using STATA version 11. Data was expressed as mean ±SD and frequency distributions. Differences between the two groups were tested using the t-test and Mann-Whitney test for normally and non-normally distributed continuous variables respectively. The Pearsons chi-squared test was used to evaluate bivariate associations between demographic and clinical variables stratified by genotype. Infant birth weight was evaluated as both a continuous and a binary (<2500g vs ≥2500g) variable. Gestational age was also evaluated as both a continuous and a binary (<37 vs v ≥ 37 weeks gestation) variable. We used multiple linear and logistic regression models with adjustment of significant covariates (marital status and exposure to passive smoking at home) determined by stepwise regression analysis, to estimate the assocation between TNF α-308 genotype and birth outcomes. All p values ≤ 0.05 were considered statistically significant.

Results

Demographic, socioeconomic and clinical characteristics of the study population are presented in Table 1. The

average age of mothers was 25.5 years. This is a relatively low socioeconomic group with most participants unemployed (67%) and single (89%). Notably, the HIV prevalence among these participants (39%) was higher than expected when compared to provincial norms. Genotypic distribution of the TNF-α genotype did not deviate from that predicted by Hardy-Weinberg equilibrium. We combined the heterozygote TNF-α GA and homozygote AA polymorphic genotype in our analyses. The polymorphic TNF-α GA+AA genotype was found among 35% of mothers (p = 0.17). The distribution of the TNF- α-308 genotype by gestational age and birth weight is given in Table 2. There was no significant difference between TNF- α genotype distribution and PTB or birth weight. The mean birth weight was significantly lower among mothers with the TNF-α AG+AA genotype (p<0.05).

Table 1: Demographic and clinical characteristics of MACE pilot (n=100) during pregnancy

Maternal characteristic	n (%)
Age (mean, SD) yrs	25.5 (6.1)
Marital status	
Married	11 (11)
Ethnicity	
African	83 (83)
Education	
Tertiary	6 (6)
Primary/Secondary Schooling	94 (94)
Maternal smoking	5 (5)
Passive Smoking at home	15 (15)
Maternal weight (mean, SD, kg) n=95	
At start of pregnancy	68.3 (17.1)
Third trimester	74.5 (17.0)
Blood Pressure (n=97)	
> 120/80 mm Hg (19%)	18 (19)
HIV positive (n=97)	37 (39)
Gestational age (mean, SD, weeks)	37 (2.7)
Birth weight (mean, SD, g)	3149 (570)

Table 2: Birth outcomes stratified by maternal TNFα-308 polymorphism (n=100)

	TNFα-308 GG	TNFα-308 GA+AA
Maternal n(%)	65 (65)	35 (35)*
Gestational age (n=92)		
>37 weeks	48 (82)	10 (18)
<37 weeks	27 (79)	7 (21)
Mean (weeks)	37.9 (3.0)	38.0 (2.2)
Birth weight		
Normal (>2500g)	54 (66)	28 (34)
Low Birth weight (<2500g)	4 (40)	6 (60)
Mean (g)	3269 (466.1)	2944 (673.1)*

*p-value<0.05

Cytokine concentrations in maternal serum stratified by TNF-α 308 genotype and birth outcomes is shown in Table 3. There was no significant difference in TNF-α levels between the wild type and the polymorphic genotype and by term and preterm deliveries. Mothers who delivered LBW infants (<2500g) showed a significantly higher mean TNF- α level compared to mothers with normal birth weight deliveries.

Table 3: Birth outcomes stratified by serum TNF-α concentration (pg/ml)

	TNF-α (mean,SD, pg/ml)
Maternal (mean,SD, pg/ml)	17.5(16.0)
TNFα-308 GG	17.3 (13.8)
TNFα-308 GA+AA	17.9 (19.6)
HIV (positive)	18.2 (17.9)
HIV (negative)	17.4 (15.2)
Gestational age (n=92)	
>37 weeks	17.2 (16.1)
<37 weeks	16.4 (15.2)
Birth weight	
Normal (>2500g)	16.3 (13.9)*
Low Birth weight (<2500g)	28.7 (27.9)

*p-value<0.05

Adjusted logistic models showed that participants with the TNF-α AG+AA polymorphic genotype were at an increased risk for PTB (OR=1.44, CI: 0.41-5.08) and LBW (OR=3.13, CI: 0.60-16.3), although this was not statistically significant (Table 4). Linear regression using continuous measurements for birth weight and gestational age showed that while mothers with the TNF-α AG+AA genotype showed a mean of 0.2 week increase in gestational age, a statistically significant drop in birth weight was evident among these mothers (β= -273.6; SE105.8; CI: -0.9-1.35).

Table 4 Multiple regression models with gestational age and birth weight stratified by genotype (n=100).

Birth Outcome	TNFα-308 GG	TNFα-308 GA+AA
Preterm <37 weeks	1.00	1.44 (0.41-5.08)
Gestational age (weeks)	Referent	0.21 (0.51) (-0.9-1.35)
Low Birth weight (<2500g)	1.00	3.13 (0.60-16.3)
Actual Birth weight (g)	Referent	-273.6 (105.8) (-0.9-1.35)*

*p-value<0.05

Models adjusted for marital status and passive smoking.

Discussion

In this pilot birth cohort, we report data on the TNF-α 308 G/A promoter polymorphism and TNF-α cytokine concentration as stratified by gestational age and birth weight. Maternal health and related birth outcomes are important public health concerns, particularly in Africa, which has a disproportionate burden of these events compared to other countries [10]. Hence, it would be prudent to search for biomarkers that would help identify potential susceptible subjects. While the TNF-α A allele was not associated with PTB, it was significantly associated with low birth weight in this study. In addition, mothers who delivered low birth weight babies had significantly higher serum concentrations of TNFα.

KwaZulu-Natal (KZN), a province which has the highest HIV prevalence in South Africa, reported a 39.5% HIV prevalence among antenatal clinic attendees in 2010. Among people presenting with HIV, 25-29 year old women have the highest HIV prevalence (32.7%) compared to other age groups [11]. We found an alarming HIV prevalence of 39% among these women, which compares with the KZN rate. The mean gestational age of 37 weeks found in this study was lower than that reported by Buchmann and Tlale [12] in a South African population. They found a mean gestational age of 39.3 weeks among 504 women (age=25.0 ± 5.8yrs) and a mean birth weight of 3190 g (± 436) which was similar to our mean (3149 g ± 570). The low mean gestational age, which borders on prematurity, is a cause for concern, particularly as this is a low socioeconomic group combined with a high HIV prevalence.

A SNP at position -308 in the promoter of the TNF-α gene (G/A) has been shown to be associated with an increase in transcription of the gene and production of the TNF-α cytokine [13]. This may lead to a systemic inflammatory response to environmental stressors although physiological changes in protein concentrations resulting from polymorphisms in TNF remain to be proven conclusively [14]. It has been shown that the frequency of the A allele at position -308 differs among different populations; 6.7% in Chinese (22), 18% among Germans (25) and 19% among British (26), 24% among Australians (27) [13]. We found a relatively higher frequency of the A allele in our sample (35%) which concurs with a study conducted among South Africans (34%) [15]. A higher frequency of this variant in a population with a high burden of HIV infection is clinically relevant as it may impact on infection.

The immune system plays an important role in pregnancy. TNF-α is an inflammatory cytokine that belongs to the subpopulation of Th1 lymphocytes. This cytokine regulates cell proliferation, differentiation and apoptosis [16]. A discrete balance between the cytokines Th1 and Th2, which are involved in fetal growth and development, determine pregnancy success. High TNF-α concentration in pregnancy has been related to the development of preeclampsia, gestational diabetes, reduced IL-10 levels, recurrent spontaneous abortions, amniotic infections and PTB [17, 18, 19, -20]. Results with the investigation of the TNF-α A allele and prematurity have been conflicting. Several authors have found significant associations between the TNF-α A allele and PTB, particularly among African American women [21] while other authors have not found any association [21,22]. While the TNF-α A allele was not associated with PTB, it was significantly associated with low birth weight in this study.

While Menon and colleagues [23] found no significant association with the A-allele and PTB, they found that TNF-α concentration was associated with PTB in African Americans but not in Caucasian Americans. Gounden et al., [15] found a significantly higher TNF-α concentration in HIV positive patients compared with controls (10.8 pg/ml and 3.6 pg/ml); as well as for the TNF-α GA genotype compared to the TNF-α GA genotype. Comparatively, maternal mean TNF-α concentration in this study was 17.5 pg/ml and did not differ significantly by genotype, gestational age or HIV status. However, the mean TNF-α concentration was significantly higher among mothers with LBW infants (16.3 pg/ml and 28.7 pg/ml). In addition, while adjusted regression models showed no association between PTB and genotype, a statistically significant decrease in birth weight was shown for mothers carrying the TNF-α A allele (β=-273.6 SE:105.8,CI:-0.9-1.35, p=0.01).

The TNF-α A allele is associated with increased transcriptional activity and carriers may have an exaggerated response to infection and a greater susceptibility to complications from infections [24,25]. These alterations in the inflammatory response may affect both gestational age and birth weight. Inflammatory cytokines like TNF-α stimulate prostaglandin synthesis in the amnion, chorion, and decidual tissue, which contributes to the initiation of labour. Furthermore, TNF-α is known to induce apoptosis, and high levels of TNF-α could damage the placenta either directly, or by activating natural killer cells, lymphokine activated killer cells, and macrophages, which in turn weakens fetal membranes. TNF-α also stimulates the production of matrix metalloproteinase enzymes, which degrades collagen, a major constituent of membranes [26, 27, 28].Thus the role of the TNF-α 308 in adverse birth outcomes is linked to functional biological alterations.

Conclusions

We found no association with TNF-α 308 polymorphism and PTB in this study. However, mothers with the variant TNF-α GA+AA genotype and with increased TNF-α levels had lower birth weight babies compared to mothers with the wild type TNF-α GG. Because of the association between this cytokine and obstetric outcomes, early identification of such cytokine alterations may prevent adverse outcomes. It must be noted that our findings are preliminary and is limited by the small sample size. However this MACE pilot study produced valuable descriptive demographic, genotypic and clinical data particularly on an important inflammatory marker from an African population. The relationship between pro-inflammatory cytokines and adverse birth outcomes such as PTB and LBW may also be modified by environmental exposures which will be explored in a subsequent study.

Acknowledgements

The authors wish to thank the participants of the MACE cohort and the nursing staff at the participating clinics and the hospitals. The Medical Research Council of South Africa (MRC) is acknowleged for funding.

Disclosure statement

The authors declare that no competing financial interests exist.

References

1. Beck S, Wojdyla D, Say L, Betran AP, Merialdi M, Requejo JH, Ruben C, Menon R, Vanlook PF. *The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity.* Bull World Health Organ 2010; 88:31–38.
2. Petrou S. *The economic consequences of preterm birth during the first 10 years of life.* BJOG 2005;112 (Suppl1), 10-15.
3. Petrou S, Mehta Z, Hockley C, Cook-Mozaffari P, Henderson J, Goldacre M. *The impact of preterm birth on hospital inpatient admissions and costs during the first 5 years of life*. Pediatrics 2003;112:1290-1297.
4. United Nations Children's Fund and World Health Organization. *Low Birthweight: Country, regional and global estimates.* UNICEF, New York, 2004.
5. Liang M, Wang X, Li J et al. 2009. *Association of Combined Maternal-Fetal TNF-α Gene G308A Genotypes with Preterm Delivery: A Gene-Gene Interaction Study*. J Biomed Biotechnol 2009:1-6.
6. Moreli JB, Ruocco AC, Vernini J, Rudge MVC, Calderon IMP. *Interleukin 10 and Tumor Necrosis Factor-Alpha in Pregnancy: Aspects of Interest in Clinical Obstetrics.* Obstet Gynecol 2011; 2012:1-5.
7. Duarte I, Santos A, Sousa H, Catarino R, Pinto D, Matos A, Pereira D, Moutinho J, Canedo P, Machado JC. *G-308A TNF-α polymorphism is associated with an increased risk of invasive cervical cancer*. Biochem Biophys Res Commun 2005; 334 (2):588-592.
8. Green MR, Sambrook, J. *Molecular Cloning. A Laboratory Manual*, 4th ed. Cold Spring Harbor Laboratory Press, 2012.
9. Wilson AG, di Giovine FS, Blakemore AI, Duff GW. *Single base polymorphism in the human tumour necrosis factor alpha (TNF alpha) gene detectable by NcoI restriction of PCR product.* Hum Mol Genet1992;1(5):353.
10. Hogan MC, Foreman KJ, Naghavi M. *Maternal mortality for 181 countries, 1980-2008: a systematic analysis of progress towards Millennium Development Goal 5.* The Lancet 2010; 375(9726):1609-1023.
11. Department of Health. 2010. South Africa HIV and AIDS statistics. 2010. Available from http://www.avert.org/south-africa-hiv-aids-statistics.htm.
12. Buchmann E, Tlale K. *A simple clinical formula for predicting fetal weight in labour at term – derivation and validation.* SAMJ 2009; 99 (6):457-460.
13. Montazeri S, Nalliah S, Radhakrishnan AK. *Association between polymorphisms in human tumor necrosis factor-alpha (-308) and –beta (252) genes and development of gestational diabetes mellitus.* Diabetes Res Clin Pract 2010; 88(2):139-145.
14. Saxon A, Diaz-Sanchez D. *Air pollution and allergy: you are what you breathe*. Nat Immunol 2005; 6(3):223-226.
15. Gounden S, Moodley D, Chuturgoon A, Karamchand L, Dawood H. *Association of -308 TNF-alpha promoter polymorphism with viral load and CD4 T-helper cell apoptosis in HIV-1 infected black South Africans.* SAJHIVMED 2012; 13(2): 72-77.
16. Patial S, Parameswaran N. *Tumor necrosis factor-α signalling in macrophages*. Crit Rev Eukaryot Gene Expr 2010; 20(2):87–103.
17. Denney JM, Nelson EL, Wadhwa PD, Waters TP, Mathew L, Chung EK, Goldenberg RL, Culhane JF. *Longitudinal modulation of*

immune system cytokine profile during pregnancy. Cytokine 2011; 53:170–177.

18. Raghupathy R. *Pregnancy: success and failure within the Th1/Th2/Th3 paradigm.* Semin Immunol 2001; 13(4):219–227.
19. Peracoli JC, Rudge MVC, and Peracoli MTS. *Tumor necrosis factor-alpha in gestation and puerperium of women with gestational hypertension and pre-eclampsia.* Am J Reprod Immunol 2007; 57(3):177-185.
20. Coughlan MT, Oliva K, Georgiou MH, Permezel JMH, Rice GE. *Glucose-induced release of tumour necrosis factor-alpha from human placental and adipose tissues in gestational diabetes mellitus.* Diabet Med 2001; 18(11):921–927.
21. Anum EA, Springel EH, Shriver– MD, Straus JF. *Genetic Contributions to Disparities in Preterm Birth.* Pediatr Res 2009; 65, 1–9.
22. Engel SAM, Erichsen HC, Savitz DA, Thorp J, Chanock SJ, Olshan AF. *Risk of Spontaneous Preterm Birth is Associated with Common Proinflammatory Cytokine Polymorphisms.* Epidemiology 2005;16(4):469-477.
23. Menon R, Velez DR, Thorse P et al. *Ethnic Differences in Key Candidate Genes for Spontaneous Preterm Birth: TNF-α and its Receptors*. Hum Hered 2006; 62:107–118.
24. Crider KS, Whitehead N, Buus RM. *Genetic variation associated with preterm birth: A HuGE review.* Genet Med 2005; 7(9):593-604.
25. Yılmaz Y, Verdi H, Taneri A. *Maternal–Fetal Proinflammatory Cytokine Gene Polymorphism and Preterm Birth.* DNA Cell Biol 2012;31(1): 92-97.
26. Maymon E, Ghezzi F, Edwin SS, Mazor M, Yoon BH, Gomez R, Romero R. *The tumor necrosis factor alpha and its soluble receptor profile in term and preterm parturition*. Am J Obstet Gynecol. 1999;181:1142–1148.
27. Raghupathy R. *TH1-type immunity is incompatible with successful pregnancy.* Immunol Today. 1997;18:478–482.
28. Watari M, Watari H, DiSanto ME, Chacko S, Shi PG, Strauss JF. *Pro-inflammatory cytokines induce expression of matrix-metabolizing enzymes in human cervical smooth muscle cells.* Am J Pathol. 1999;154:1755–1762.