# The cytokine TNF-alpha

## Genetics and suitability for prenatal risks' assessment

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## ABSTRACT

The tumor necrosis factor (TNF-α) is a cytokine known as a mediator of inflammation and immunity. The genes coding the tumor necrosis factors alpha and beta are considered part of class III major histocompatability complex. The 2 involved genes have been mapped to chromosome 6. Certain mutations in the TNF-α gene are believed to be causative for increased production of the cytokine. In this respect, the most common variant is the TNF2 allele, a single nucleotide substitution of guanine by adenine at position -308 relative to the promoter transcription site of the gene. Elevated production of TNF-α has been found to be associated with several infectious diseases including malaria. Elevated levels of TNF-α have also been observed to associate with increased risk of preterm delivery, chorioamnionitis and fetal morbidity including encephalopathy. The present article reviews the genetics of the cytokine TNF-α and discusses its suitability as a candidate marker for assessment of increased risk of preterm delivery and fetal morbidity.


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The tumor necrosis factor alpha (TNF-α) is a cytokine produced by activated macrophages and it is considered a critical mediator of inflammation and immunity. When produced in excess, TNF-α has been considered a likely candidate molecule for the link between maternal intrauterine infection, preterm delivery, and fetal morbidity.[1] Dammann et al[2] suggested that maternal and fetal genetic factors, involved in the regulation of TNF-α production, influence a woman's infection-associated risk of giving birth before term and her infant's risk of disease including encephalopathy. These concepts have provoked an interest in the clinical significance of TNF-α and set the scene for further studies to investigate its potential value in assessment of increased risk of preterm delivery and fetal morbidity. It is known that preterm delivery is not a homogeneous obstetrical entity; and therefore, several categories of preterm delivery may be distinguished. In most populations, the majority of preterm births are the result of spontaneous onset of contractions in the absence of any antecedent pathologic features; this subset is termed "idiopathic preterm delivery". A second subset is when delivery is traumatic or pathologically induced like in the cases with precedent spontaneous rupture of membranes with an infectious etiology. Multiple-gestation may be considered a third category. Finally, teenage pregnancy, congenital abnormality of the uterus, and other factors such as stress, smoking, illegal narcotic drug use, especially cocaine, may be considered a fourth category. In spite of the progressive technical advances, figures from the United States of America (USA) indicate that both the preterm delivery rate (10%) and the rate of

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low-birth-weight infants (7%) have not decreased significantly.[3] The present article is intended to throw some light on the genetic predisposition to preterm delivery and fetal morbidity. I shall focus on the genetics and clinical implications of the cytokine TNF-α and the relationship between elevated levels of TNF-α on one hand and preterm delivery and fetal morbidity on the other. The current literature on the topic will be reviewed, and evidence in support of involvement of the TNF-α with preterm delivery and fetal white matter damage will be cited. This will then be followed by a conclusive comment on the suitability of the level of TNF-α, and mutations in the TNF-α gene, as candidate markers for assessment of the risks of preterm delivery and fetal morbidity.

***Genetic predisposition to preterm delivery.*** Several observations have been noticed for a genetic predisposition to preterm delivery. First, a leading risk factor for preterm birth is having a prior pregnancy ending in a premature delivery.[4-6] This risk significantly increases with 2 or more prior preterm deliveries. Carr-Hill and Hall[6] looked at the obstetric outcomes of 6072 Scottish women and showed that with a history of one prior preterm birth a 15% recurrence risk of a subsequent preterm birth existed. With a history of 2 prior preterm births, the risk of a subsequent preterm birth was more than doubled at 32%. Second, there are well-described racial differences in the rate of preterm deliveries. Women of African origin had twice the rate of preterm delivery compared with Caucasian women even when confounding social and economic variables are controlled.[7-9] Third, analysis of data of preterm delivery in the USA, by Porter et al,[10] where extensive genealogy records were available, suggested a familial tendency for preterm delivery. Because all the above-cited observations clearly imply 'intrinsic' cause of preterm labor, Porter et al,[10] along with others,[11] hypothesized that genetic predisposition could be responsible for many cases of preterm delivery.

***Preeclampsia and perinatal complications - Saudi findings.*** Generally, several perinatal complications, such as preterm delivery and fetal morbidity, have been observed to be associated with preeclampsia (PE). Despite the eminent clinical significance of this topic, published results are relatively scarce in the Saudi community. Two examples of works in this community are chosen due to its relevance. First, from their study in the Northwestern region of Saudi Arabia, Lawoyin and Ani[12] have reported that PE attained a hospital-based incidence rate of 2.8%, and that women at extremes of maternal age, the nulliparous and high parity women, women with a high body mass index, and those with late or no antenatal care were at greater risk of developing PE. Second, in a 10-years study involving 27787 women from the Eastern region of Saudi Arabia, a hospital-based incidence rate of PE of 2.5% with a high proportion of PE cases occurring among the nulliparous women and those at the extreme ends of the reproductive age (<20 and >40 years) have been reported by Al-Mulhim et al.[13] Preeclampsia complicated pregnancies were positively associated with high need for induction of labor, more premature deliveries and low birth-weight of infants. The perinatal outcome of maternities with PE shows that stillbirths (2.3%) and early neonatal deaths (1%) comprised an overall mortality rate of 33.6 per 1000. Preterm deliveries comprised 13.5% in women without PE, but 30.2% in women with PE. Placental abruption was encountered at 12.6% of the latter group. The incidence of encephalopathy in newborns from mothers with PE comprised 1.9%. The association of incidence and complications of PE with a polymorphic variant of the TNF-α gene, for example, the TNF2 allele, was not addressed in either one of the cited papers.

***Genetics of the cytokine tumor necrosis factor alpha.*** The TNF-α locus is considered part of class III major histocompatibility complex that contains genes for the TNF-α and ß on chromosome 6p.[14,15] A single nucleotide polymorphism, from guanine (G) to adenine (A), at position -308 relative to the transcription start site in the promoter region of the TNF-α gene, constitutes the TNF2 variant allele whereas the normal allele is referred to as TNF1. This promoter-region polymorphism has been described as causative for the higher constitutive and inducible levels of TNF-α gene transcription and subsequently increased TNF-α production.[16-19] The TNF2 allele is in linkage disequilibrium with some human leukocyte antigen (HLA) class I and II antigens.[20] Subsequent studies have, however, shown that TNF2-associated deaths among cerebral malaria[18] and septic shock patients[21] were independent of linked HLA alleles. Apart from the TNF2 (-308 allele), there are at least 2 other single nucleotide polymorphisms in the TNF-α gene promoter region. The TNF -238A allele was found to be associated with severe anemia due to malaria[18] and the TNF -376A allele was associated with cerebral malaria.[22] However, TNF2 is not in linkage with any of these 2 polymorphisms.[22,23] The TNF2 is an allele variant of the TNF-α gene with an overall frequency of 16% found in 2 populations.[16,18] More direct evidence of elevated TNF-α is associated with yet another type of polymorphism. This polymorphism, TNFB2, occurs 1 Kb upstream from the TNF-α gene in the gene encoding TNF-ß, a related cytokine. Interestingly, while TNFB2 does not seem to affect the production of TNF-ß itself, it is associated with higher TNF-α synthesis[24,25] and an increased risk of adverse outcome in patients with severe infections.[25] Among patients with severe

sepsis, mortality was 88% in TNFB2 homozygous individuals, 37% in heterozygous patients, and 25% in those who did not have the TNFB2 allele at all.[25] These and other findings suggest that the genetic variability of the TNF locus may contribute to the susceptibility to, or the outcome of, various autoimmune, neoplastic, and infectious diseases.[26]

***Clinical implications of elevated tumor necrosis factor alpha.*** The TNF-α is a pro-inflammatory cytokine known to be essential in the control of many intracellular infectious agents in humans.[27] It is a critical mediator of inflammation and immunity as illustrated by inability of mice deficient in the receptor for this cytokine to control *Listeria monocytogenes* infection but are resistant to endotoxic shock.[28] Elevated levels of TNF-α have been implicated in the pathogenesis of infectious diseases such as malaria,[29] meningococcal disease,[30,31] schistosomiasis[32] and some autoimmune diseases.[26] The TNF2 allele, that causes increased levels of TNF-α, was also found to be associated with lepromatous leprosy,[33] scarring trachoma infections,[34] mucocutaneous leishmaniasis,[35] and a high risk of death in meningococcal disease.[36] Homozygosity for this rare TNF2 allele has been associated with a 4 fold increased risk of cerebral malaria and 7 times greater likelihood of death after a cerebral malaria attack.[18] Furthermore, prenatal exposure to elevated TNF-α has been suggested to be a risk factor for respiratory distress syndrome[37] and fetal septic shock.[21] The pathogenic mechanism underlying association of the TNF2 allele with increased infant mortality among those born prematurely is currently not clear. It is possible that premature children born with TNF2 may be at a high risk of developing life-threatening complications such as pneumonia, septic shock, or other complications of unknown etiology, especially when they have respiratory or other organ systems that are not fully developed at birth. This hypothesis is consistent with the observation that prematurely born infants homozygous for TNF2, had several-fold increased risk of death compared with TNF1/TNF2 heterozygotes.[38] Hence, the TNF2 allele seems to be associated with severe disease caused by a variety of infectious agents. Similar to the original suggestion of Dammann et al,[2] a plausible hypothesis that abnormal levels of TNF-α could trigger a pathway that eventually leads to infant mortality has been proposed by Aidoo et al.[38] Grau and Maenel[39] likewise emphasized the importance of TNF-α as part of the natural immunologic response to systemic infection and the potential risks of blocking TNF-alpha actions.

***How is tumor necrosis factor alpha related to preterm delivery and white matter damage.*** Infants of extremely low gestational age or birth weight, or both are at prominently increased risk of white matter damage.[40] Deprivation of maternally provided trophic influences during a vulnerable period of myelinogenesis might account for a portion of this increased risk.[41] On the other hand, correlates of intrauterine infection are also associated with an increased risk of white matter damage[41-45] and cerebral palsy among very low weight infants.[46-50] Several research teams have obtained evidence that TNF-α could be involved as one of the factors that contribute to white matter damage. Examples of these studies are listed as follows: a) amniotic fluid TNF-α levels were found to be significantly higher among infants who later developed signs of white matter damage on neonatal cranial ultrasound images and subsequently developed cerebral palsy than among their unaffected peers.[51] 2) Median TNF-α levels were observed to be higher, although not statistically significantly, in umbilical cord plasma of newborns with defined white matter damage on ultrasonography than in controls.[52] 3) In 3 postmortem studies, brains with white matter damage were more likely than brains without white matter damage to have TNF-α immuno-reactivity.[53-55] 4) Among preterm infants, TNF-α might contribute to systemic hypotension, mediate intravascular coagulation, damage endothelial and ependymal cells and neuralgia, and induce the synthesis of potentially harmful substances.[1,45] Both development of the neonatal brain and white matter disease in preterm delivery are probably due to multiple factors that include increased production of TNF-α and delayed myelination or dysmyelination. Further studies are warranted to assess the exact role, and the mechanism, of increased TNF-α on white matter damage.

***Association of tumor necrosis factor 2 allele with preterm delivery and neonatal morbidity.*** Many studies have shown that increased TNF-α levels in placental and fetal tissues are associated with spontaneous abortions and preterm deliveries.[56-60] Elevated TNF-α concentrations in the amniotic fluid were also associated with preterm labor and delivery.[61-66] In a recent study, in a total of 1048 Kenyan children, the genotype frequencies of the TNF1 (normal) and TNF2 (variant) alleles were reported to be 0.90 and 0.10, TNF2 homozygosity was associated with more preterm deliveries when compared with TNF1 homozygotes (rate ratio [RR] 7.3; $p$=0.002) and TNF1/TNF2 heterozygotes (RR 6.7; $p$=0.008). Furthermore, among children born prematurely, the TNF2 allele was significantly associated with a higher risk of death in infancy compared with TNF1 (RR 4.47), and the risk of death was higher among homozygotes than among heterozygotes.[38] Despite the confirmed association, TNF2 may only be causally associated with infant mortality. Other

closely linked genetic factors may play a role. In a recent study, Simhan et al[67] have similarly observed in their retrospective study that chorioamnionitis was present in 12.1% of women who had spontaneous labor from 37 to 42 weeks' gestation. Among women who did not carry the TNF2 allele the chorioamnionitis rate was 7.4%, whereas among women who carried this allele the chorioamnionitis rate was 24.4%. Hence, carriage of the TNF2 allele is associated with a more than 3 fold increased risk of chorioamnionitis even when accounting for important clinical and microbiological risk factors.[67] Likewise, Roberts et al[68] reported that the maternal TNF2 allele was significantly associated with preterm delivery due to premature rupture of membranes and not with idiopathic preterm delivery. However, Dizon-Townson et al[69] in a previous study concluded that in neonates and mothers the TNF1/TNF2 heterozygous allele was not positively associated with preterm delivery. A premature rupture of membranes is well known to be associated with an infectious etiology in many cases. In this context the findings of Roberts et al[68] and Aidoo et al[38] were consistent with the hypothesis that the increased TNF-α production, due to TNF2 allele, is initiated by maternal intrauterine infections that cause premature rupture of membranes and lead to preterm delivery. Tumor necrosis factor 2 homozygosity was similarly found to be associated with a 2 fold increased risk of low birth weight.[38] Although this increased risk was not a significant one, this observation is consistent with the fact that preterm delivery is often associated with a low birth weight.

***Fetal inflammatory response is an important factor in both preterm delivery and white matter damage.*** The obvious possibilities for the origin of the TNF-α found in the amniotic fluid are: TNF-α could stem either from the fetus, the mother, or from both. The presence of TNF-α in the fetal circulation and the fetal brain does not preclude a maternal contribution, since cytokines are produced in the placenta and can cross the blood-brain barrier.[45] In any case, it seems most likely that a strong fetal inflammatory response is a crucial contributor to both preterm labor and white matter damage in the preterm newborn. Evidence for this notion has been drawn from the following elegantly documented observations: First, the concentration of pro-inflammatory cytokines in amniotic fluid increases with increasing grade of histological chorioamnionitis in preterm delivery.[70] Second, the fetal, but not maternal, cytokine levels correlate with the severity of histologic umbilical vasculitis. This indicates a fetal inflammatory response even closer to the fetus than chorioamnionitis.[71] Third, the onset of spontaneous preterm delivery is preceded by pronounced elevation of pro-inflammatory cytokine response in samples from the human fetus obtained by cordocentesis.[72-74] Fourth, elevated neonatal cytokine levels in umbilical cord predict white matter damage.[52] Fifth, histologic expression of TNF-α appears to be associated with periventricular leukomalacia,[53,55] a focal form of white matter damage in preterm newborns.[41] Although much of this work focuses on the role of interleukin-6, Levinton[1] and Dammann and Leviton[75] consider interleukin-6 more likely a systemic marker of an inflammatory response, while the characteristics of TNF-α make it more likely a local contributor to white matter damage.

Discussion. The close associations between preterm-delivery, neonatal and maternal morbidity have been well recognized. The underlying mechanisms of pathogenesis have and continue to be the focus of attention of investigators worldwide. Thus, studies on the predisposing factors, including epidemiological and genetic factors, and other intrinsic factors like the role of cytokines and interleukin, are currently being undertaken. Such studies are desirable since they may provide hints that bear significance in the pediatrics and obstetrical practice. Although many studies have shown the association of elevated TNF-α with maternal and fetal morbidity after infection with different pathogens, the exact mechanistic impact of this cytokine still deserves to be studied. Similarly, the impact of TNF-α on fetal and maternal outcome, including outcome of pregnancies complicated with PE (and eclampsia) warrants detailed investigations at the molecular level. The results of such studies bear the potential benefit of assessment of the risk of preterm delivery and fetal white matter damage. Genetic studies involving the TNF-α gene and its phenotypic expression, in maternal and fetal material, are warranted. In particular, it would be interesting to study the association between the TNF-α gene variant -308G>A, with preterm delivery and occurrence of cerebral palsy. It will also useful to document the prevalence of TNF2 allele in the Saudi community taking into consideration the relatively high rate of consanguinity in this population.

In conclusion, evidence has been obtained in support of involvement of elevated levels of the cytokine TNF-α with pregnancies complicated with infection. Therefore, the cytokine TNF-α, and polymorphic variants of the gene controlling its synthesis, for example the TNF2 allele, seem to be suitable candidate markers for assessment of an increased risk of preterm delivery and fetal morbidity. Genetic studies, coupled with controlled prospective clinical studies in mothers and infants, are recommended in order to evaluate the efficacy of TNF-α in the assessment of prenatal risks.

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