Molecular Immunology 97 (2018) 82–93

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Role of IL-1β, IL-6 and TNF-α cytokines and TNF-α promoter variability in *Plasmodium vivax* infection during pregnancy in endemic population of Jharkhand, India

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ARTICLE INFO

Keywords:
Inflammatory cytokines
Vivax malaria
Promoter Polymorphism
phism
MIP
Jharkhand

ABSTRACT

Background: The combinatorial effects of *Plasmodium* infection, perturbation of inflammatory responses and the dichotomic role of TNF promoter polymorphism has potential clinical and physiological relevance during pregnancy.

Objective and Methods: This coordinated orchestration instigated us to investigate the circulating level of inflammatory cytokines (IL-1β, TNF-α and IL-6) employing ELISA in a stratified group of samples and the plausible genetic association of TNF-α −308 G/A using PCR-RFLP/sequencing during *Plasmodium vivax* infection in pregnancy.

Results: We observed significantly elevated concentrations of IL-1β were observed, followed by IL-6 and TNF-α in women with malaria (WWM) and in malaria in pregnancy (MIP). Further, elevated IL-1β, followed by TNF-α and IL-6 were detected in the non-infected pregnancy group. The differential dynamics of inflammatory cytokine concentration during each trimester of pregnancy with and without *P. vivax* infection were detected. For the first time, a high level of IL-6 was observed in the first trimester of MIP and high IL-1β in healthy pregnancies. In the second trimester, however, we observed a high level of IL-1β in the MIP group compared to a sustained high level of IL-1β in the healthy pregnancy group. In the third trimester, high IL-1β was sustained in the MIP group and healthy pregnancies acquired a high TNF-α level. The genotypic distribution for the TNF-α promoter −308 G/A position was observed to be nonsignificant and mildly associated during MIP (OR = 1.4) and in WWM (OR = 1.2). Moreover, based on genotypic distribution, we observed a well-correlated and significantly elevated TNF-α concentration in the mutant homozygote genotype (AA; $p = 0.001$) followed by heterozygotes (GA; $p = 0.0001$) and ancestral genotypes (GG; $p = 0.0001$) in both MIP and WWM subjects.

Conclusion: The observation of elevated IL-1β and IL-6 in MIP and TNF-α in WWM may be regarded as a prognostic inflammatory marker of infection and pregnancy. Most particularly, the TNF-α concentration and its polymorphic variability in the promoter region may indicate genetic susceptibility and mildly influence the risk for *P. vivax* infection during pregnancy and in women with malaria.

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https://doi.org/10.1016/j.molimm.2018.03.019
Received 17 October 2017; Received in revised form 18 March 2018; Accepted 22 March 2018

1. Introduction

Pregnant women and their infants are more susceptible to common and preventable infectious diseases, including malaria, and most of this disease's mortality and morbidity is borne by children and pregnant women. With more than 300 million people suffering from malaria and over 50 million women exposed to the risk of malaria during pregnancy, and an annual infant fatality of 75,000–200,000, malaria is a serious public health concern across the globe (Desai et al., 2007). The dominant malaria parasites infecting humans, *Plasmodium falciparum* and *Plasmodium vivax*, contribute to pregnancy-associated malaria and cause various adverse pregnancy outcomes depending upon the clinical mediators and geographical locations (Dellicour et al., 2010). Both infections have unique features of pathogenesis, epidemiology and clinical course during pregnancy given that parasites can sequester in the placenta, reduce maternal hematocrit, and can cause massive inflammation most particularly at the maternal–foetal interface. Moreover, pregnant women have a lower resistance to malaria; in fact, they are four to 12 times more likely to have patent parasitaemia and are more susceptible to severe complications of the disease compared to other adults (Brabin, 1991).

Infection-induced physiological perturbation and immunological imbalance affects the overall inflammatory equilibrium of women's immune system necessary for a successful, full-term pregnancy. The counter balancing phenomena of anti- and pro-inflammatory cytokines is potent factor in the regulation of an effective immune response to malaria as well as role in supporting successful pregnancy via eliciting the coordinated orchestration of innate and adaptive immunity (Chene et al., 2014; Yasnot et al., 2013). More precisely, inflammatory cytokines have a dichotomic role in human immune responses to malaria disease and during pregnancy, although the balance and perturbation of inflammation alone and in conjunction with parasitic infection is less clearly defined and elucidated in humans. In pregnancy, uterine decidual macrophages (also called M1 macrophages) induce the development of an inflammatory phenotype that is characterized by elevated secretion of inflammatory cytokines (Nagamatsu and Schust, 2010). During a normal pregnancy, the Th1/Th2 activity balance is strongly shifted toward Th2 activity and it plays a potentially protective role in the foetal–maternal relationship (Souza et al., 2013; Sykes et al., 2012a). Inflammatory and infection processes alter the balance of Th1 and Th2 cytokines, causing a shift towards a Th1 predominance, which initiates and intensifies the cascade of inflammatory cytokine production involved in spontaneous abortion, preterm delivery, preeclampsia and labour (Chaouat et al., 2002). However, a delicate balance between pro-inflammatory and anti-inflammatory cytokines regulates inflammatory kinetics during pregnancy (Yilmaz et al., 2012). The regulated interplay between pro- and anti-inflammatory cytokines is a pivotal factor in determining malaria parasitaemia, birth outcome, clinical protection and rate of recovery (Riley, 1999), while overproduction contributes to adverse birth outcome, pregnancy-associated complications, immunopathology and disease progression (Umbers et al., 2011). Pro-inflammatory cytokines play a key role in orchestrating the complex events involved in inflammation and immunity (Peeters et al., 2001). Previously, it has been demonstrated that an elevated level of inflammatory cytokines such as TNF-α, IL-1β and IL-6 (Friedland et al., 1993) was associated with disease severity in malaria during pregnancy (Brickley et al., 2015; Day et al., 1999). These cytokines are instrumental in influencing the killing and clearance of parasites from the placenta by enhancing the phagocytic activity of macrophages, generating reactive oxygen intermediates and L-arginine-derived nitric oxide, and stimulating the proliferation of T cells. Therefore, Th1-type responses are of parasitological importance. However, overproduction can threaten normal pregnancy, as the Th1 response is associated with maternal anaemia, spontaneous abortions and premature deliveries (Raghupathy, 1997).

The parasite's selective pressure on the human genome and the advent of molecular genetics has opened evidence-based approaches to numerous undisputed polymorphic loci that appear to contribute to malaria susceptibility or resistance and polymorphism-associated variability of malaria phenotypes (Weatherall and Clegg, 2002). Based on well-documented evidence, various TNF-α gene promoter loci have been assessed in disease-association studies (Gichohi-Wainaina et al., 2016; Olaniyan et al., 2016). Among the single nucleotide polymorphisms of TNF-α gene promoter at position −308, a G/A substitution has been shown to be associated with pregnancy (Stonek et al., 2007) and delivery outcome (Reddy et al., 2014) in various diseases, including in patients with severe malaria anaemia, asymptomatic malaria and cerebral malaria (Gounden et al., 2012). The established sensitivity of *Plasmodium* parasites towards inflammatory responses and the implication of a disturbed inflammatory network have attracted scientists to investigate inflammatory cytokines as a potential candidate in the diagnosis and monitoring of malaria-induced clinical complications and disorders during pregnancy.

Importantly, we have limited knowledge and understanding about the orchestration of inflammatory cytokines profile, TNF-α transition polymorphism in *Plasmodium* vivax-infected malaria patients during pregnancy and without pregnancy, globally and particularly from the perennially endemic transmission zone i.e. Hazaribag, Jharkhand, India. Considering the obstetric significance and immunomodulatory role of inflammatory responses during host-parasite interaction; we systematically investigated the circulating concentration of IL-1β, TNF-α and IL-6 in a clinically stratified group of subjects i.e. malaria during pregnancy, women with malaria without pregnancy, and in different trimesters of pregnancy with and without malaria infection attending an antenatal care unit (ANC) at Sadar Hospital, Hazaribag, Jharkhand, India as compared to region and age matched healthy women subjects. Additionally, we also investigated the transition polymorphism at −308 region of TNF promoter and its association with malaria in pregnancy; and to the best of our knowledge, there has been no published study examining the association and such comprehensive inflammatory profile on Indian isolates from the endemic region of Jharkhand. The rationale behind the present investigation was to explore the clinical spectrum of inflammatory interplay, host–parasite interaction, and to evaluate the potential of TNF-α, as prognostic immuno-genetic marker during infection and pregnancy from endemic population.

2. Methods

2.1. Study sites and population

A cross-sectional investigation was conducted in the ANC units of Sadar Hospital in Hazaribag district of Jharkhand, India (Fig. S1), a rural-cum-semi urban district, an eastern Indian state located in the tropical and forested zone with favourable geoclimatic and ecological conditions conducive to rich and distinctive flora and fauna biodiversity, considered to be a malaria-endemic area in the state of Jharkhand. Endemically, the study site has significance in view of low but perennial and asymptomatic transmission of malaria throughout the Hazaribag district with an average slide positivity rate (SPR) of 8.7% over the last 3 years (State Malaria Control Program Annual Report Ranchi, Jharkhand, Directorate of Health Services, 2010), with the burden of malaria mainly due to *P. vivax* in the majority of the indigenous population, a mix of tribals, Scheduled caste, Scheduled tribes and other casts; exceptionally, typical social stratification includes gender disparity (Sohail et al., 2015). The region is highly endemic for *vivax* malaria lacerated with women health issues including malaria in pregnancy (Sohail et al. 2015, Hamer et al., 2009) and to the best of our knowledge, such profile and clinical correlation has not been investigated before on Indian clinical isolates.