# Pro-Inflammatory Cytokine Profile of Pregnant Women with Asymptomatic Malaria Parasitemia in Uyo, Nigeria

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## ABSTRACT

***Malaria is still regarded as a public health problem in tropical Africa and is endemic in Nigeria. Pregnant women and their fetuses are at greater risk of malaria in endemic areas. Pro-inflammatory cytokines induced by malaria parasites play vital role in pregnant women's immunity to malaria. The aim of this study was to investigate the level of Interferon-gamma (IFN-γ) and Tissue Necrosis Factor α (TNF-α) in asymptomatic pregnant women with and without malaria parasite infection in Uyo, South-South Nigeria. Blood samples of asymptomatic pregnant women attending the antenatal clinic at the University of Uyo Teaching Hospital Uyo and St. Luke's Hospital, Anua, Uyo, were analysed for malaria parasites by microscopy of blood films and for IFN-γ and TNF-α using sandwich ELISA methods. Ninety-six pregnant women were recruited of which 84 (87.57 %) were positive for Plasmodium falciparum. The mean levels of IFN-γ and TNF-α among malaria-infected women were significantly higher than levels among uninfected pregnant women. Pregnant women with asymptomatic parasitemia have higher levels of IFN-γ and TNF-α and may be at risk of fetal morbidity due to increased levels of cytokines released as a result of malaria parasite infection.***

***Keywords: Cytokines, Asymptomatic malaria, Pregnancy, Nigeria***

## INTRODUCTION

Malaria remains a leading cause of mortality and morbidity in sub-Saharan African countries including Nigeria. Globally in 2021, there were an estimated 247 million malaria cases in 84 malaria-endemic countries which represented an increase of 2 million cases compared with 2020 and most of the increase in case numbers occurred in countries in Africa. Nigeria is the country with the highest number of cases of malaria globally and accounts for 38.4% of global malaria deaths in children aged under 5 years.[1]

There has been an increase in exposure to malaria in pregnancy in Africa. This increased exposure is estimated to have resulted in 961,000 neonates with low birth. Low birth weight is a strong risk factor for neonatal and childhood mortality.[1]

Pregnant women and their foetuses are at higher risk of malaria and that has been the cause of 20 per cent of maternal deaths in endemic areas. One of the main pathophysiological events during malaria infection are the excessive production and release of pro-inflammatory cytokines. Infected RBCs in the placenta cause an inflammatory environment which increases inflammatory cells and cytokines that are deleterious to the placenta.[2] Innate immune cells that produce pro-inflammatory cytokines are majorly mononuclear phagocytes such as macrophages and dendritic cells, although they can also be produced by natural killer (NK) cells, endothelial cells and mucosal epithelial cells.[3]

The pro-inflammatory cytokines such as tumour necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), and interleukin-12 (IL-12) aid in inhibiting parasite growth and stimulate monocyte phagocytosis to enhance clearance of parasitized erythrocytes. Inflammatory cytokines such as IL-17 and IL-22 contribute to inflammation by recruitment of neutrophils and induction of secretion of several pro-inflammatory cytokines. The timely regulation by anti-inflammatory cytokines such as IL-10, IL-4, and IL-13 to control the production and possible cytopathic effects of pro-inflammatory cytokines, plays a major

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role in limiting the progression of uncomplicated malaria into its severe forms.

The altered physiology and immunity during pregnancy are responsible for severe malaria in pregnant women. The increased concentration of cortisol, during pregnancy makes women more susceptible to malaria by directly inhibiting natural killer cell activity against P. falciparum-infected erythrocytes. The cell-mediated immunity is depressed to support the development of the placenta and the fetus. But this halt in cell-mediated immunity, however, makes pregnant women more susceptible to intracellular pathogens.[5] The effects of elevated levels of pro-inflammatory cytokines are oxidative stress, decrease in heat shock protein expression and apoptotic cell death in placenta, leading to poor pregnancy outcomes such as miscarriage, stillbirth and low birth weight.

There have been reports of increased levels of IFN- γ in malaria-infected pregnant women in Edo State in South-South Nigeria6 and increased levels of IFN-γ and TNF-α in malaria-infected women in Abia State in South-Eastern Nigeria.[7] The aim of this study was to investigate the level of IFN-γ and TNF-α in asymptomatic pregnant women with and without malaria parasite infection in Uyo, South-South Nigeria.

## MATERIALS AND METHODS

### Study area and participants

This study was carried out in Uyo, southern Nigeria. There is high malaria transmission throughout the year with peak levels during August through October. Pregnant women who were on their first antenatal visit to the antenatal clinic at the University of Uyo Teaching Hospital (UUTH) and St. Luke's Hospital, Anua (SLHA) and reported no symptoms of malaria were recruited into this study between September and December 2019. Blood samples were obtained from consenting participants for malaria parasite detection and measurement of cytokines.

### Malaria parasite detection

Malaria parasite identification and quantification was carried out using thin and thick films prepared following WHO protocol. A parasite count was performed by a certified malaria microscopist. Parasitemia was classified as mild if the parasite count ranged from 100 to 1,000 parasites/µL, moderate if the parasite count ranged from 1,000 to 10,000 parasites/µL and severe if above 10,000 parasites/µL.7

### Cytokine levels

Commercial sandwich ELISA reagents from Bridge Biotech Ltd, Palo Alto, USA were used to determine levels of IFN-γ and TNF-α in the plasma of participants and the protocols were strictly adhered to.

### Statistical analysis

Data was analyzed using SPSS version 24 (SPSS, Inc, Chicago, IL, USA). Means of cytokine levels for different classes of participants were presented in tables. Student's t-test was used to determine the association between cytokines levels and other variables.

## RESULTS

A total of 96 women were recruited into this study with 32 (33.3%) in the first trimester of pregnancy, 47 (48.9%) in the second trimester and 17 (17.7%) in the third trimester. The mean age of participants was 28.34 ± 7.35 years. Most of the participants (49/96) were multigravidae malaria parasitem. There were 12 participants who were negative for malaria parasites. Of the malaria positive participants, 52 had mild parasitemia while 32 had moderate parasitemia. There was no participant with severe parasitemia.

### IFN–γ and TNF–α levels

The mean IFN–γ and TNF–α levels of different classes of participants and their association with malaria parasitemia is shown in Table 2. The highest levels of both cytokines were seen in malaria-positive women, women in their second trimester of pregnancy and primigravid women. The association of mean cytokine levels with levels of parasitemia showed higher levels with moderate parasitemia compared to mild parasitemia.

Table 1: Characteristics of participants and controls– age, trimester, parity, malaria parasitemia, IFN-γ and TNF-α levels

| **Characteristic** | | **Malaria positive** | **Malaria negative** |
|---|---|---|---|
| Age/age range | Mean | 28.32±7.29 | 28.50±7.78 |
| | <20 | 12 | 2 |
| | 21-30 | 42 | 6 |
| | 31-40 | 19 | 2 |
| | >40 | 11 | 2 |
| Parity | Primigravida | 35 | 5 |
| | Multigravida | 49 | 7 |
| Trimester | 1 | 27 | 4 |
| | 2 | 44 | 4 |
| | 3 | 13 | 4 |
| Malaria parasitemia | 0 | 0 | 12 |
| | Mild | 52 | 0 |
| | Moderate | 32 | 0 |
| | Severe | 0 | 0 |

Table 2: Association of cytokines with trimester and parity levels in malaria-positive and negative women

| **Trimester** | **Cytokine** | **Malaria positive** | **Malaria negative** | ***P*** |
|---|---|---|---|---|
| First trimester | IFN – γ | 19.85 ± 14.81 | 5.37 ± 3.04 | 0.064 |
| | TNF – α | 378.27 ± 83.93 | 153.62 ± 18.44 | 0.000 |
| Second trimester | IFN – γ | 21.12 ± 12.13 | 5.25 ± 2.82 | 0.013 |
| | TNF – α | 301.53 ± 53.87 | 184.33 ± 13.10 | 0.000 |
| Third trimester | IFN – γ | 19.69 ± 10.60 | 4.97 ± 3.94 | 0.017 |
| | TNF – α | 298.13 ± 48.07 | 157.21 ± 14.01 | 0.000 |
| Primigravida | IFN – γ | 22.60 ± 0.92 | 4.15 ± 1.03 | 0.000 |
| | TNF – α | 394.43 ± 46.43 | 182.72 ± 20.81 | 0.000 |
| Multigravida | IFN – γ | 19.03 ± 2.81 | 4.32 ± 2.72 | 0.000 |
| | TNF – α | 328.62 ± 60.37 | 151.32 ± 12.41 | 0.000 |
| Total | IFN – γ | 20.52 ± 2.02 | 4.25 ± 2.02 | |
| | TNF – α | 356.04 ± 54.56 | 164.40 ± 15.91 | |

Table 3: Immunological parameters in relation to Parasite levels

| | **Mild parasitemia** | **Moderate parasitemia** | **High parasitemia** | ***P*** |
|---|---|---|---|---|
| IFN – γ | 17.83 ± 12.09 | 76.82 ± 50.13 | - | 0.000 |
| TNF – α | 284.72 ± 44.34 | 421.63 ± 28.21 | - | 0.000 |

## DISCUSSION

Malaria is still regarded as a public health problem in tropical Africa. Pregnant women constitute one of the more vulnerable populations to malaria. We observed a very high prevalence of asymptomatic malaria parasitemia among pregnant women who participated in this study. Pregnant women are at greater risk of malaria infection and of asymptomatic malaria disease than non-pregnant adults[8] and in a study in Bangladesh, asymptomatic malaria parasitemia was much more common in pregnant women than in non-pregnant adults.[9] Asymptomatic malaria infections have a significant impact on malaria transmission in different populations and settings. A review of studies on asymptomatic malaria infections in pregnancy put the global frequency among women analyzed at 10.8%.[10] While asymptomatic malaria parasitemia has been linked to the protection of children from developing severe disease, it has also been implicated in the persistence of parasite transmission in endemic areas.[11]

Although previous studies have shown high levels of cytokines among pregnant women with malaria parasites in Nigeria and elsewhere, this study has demonstrated high levels of cytokines in women with asymptomatic malaria parasitemia. These levels were significantly higher than those with no parasitemia or submicroscopic parasitemia. This increase was seen in participants in all trimesters of pregnancy. Studies have suggested that cytokines like TNF-α may mediate the effects of malaria in pregnancy particularly intrauterine growth restriction and preterm delivery, both of which contribute to the delivery of low birth-weight infants.[12] There appear to be different mechanisms by which malaria causes preterm delivery and intrauterine growth restriction, as increases in inflammatory cytokines such as TNF-α and IL-8 are associated with intrauterine growth restriction but not with preterm delivery due to malaria.[5] Furthermore, asymptomatic malaria infections are strongly associated with maternal anaemia[9] which has a further impact on infant wellbeing. These findings raise concern about the potential effect of high levels of cytokines on infants even in the absence of symptomatic malaria disease.

Some of the mechanisms proposed to explain increased cytokine levels in malaria are phagocytosis of the parasite or the haemozoin, glycosylphosphatidylinositol (GPI) and the parasite toxin leading to up-regulation of cytokines,[13,14] generation of reactive oxygen species such as hydrogen peroxide ($H_2O_2$), hydroxyl (OH- ) and superoxide (O2-) during oxidative stress which activates leucocytes with the release of more cytokines, [15] activation of coagulation factors in response to endothelial wall damage occasioned by sequestration and adherence of the parasite.[16] The coagulation factors, together with other plasma proteins, are also inflammatory mediators and elicit the release of inflammatory mediators including cytokines.[15]

We found cytokine levels declining in later trimesters of pregnancy. Proinflammatory cytokine levels correlate with malaria parasite levels. Malaria in pregnancy peaks between 13 and 16 weeks and declines toward term. In a study in Saudi Arabia, higher parasite levels were also found earlier in pregnancy.[17] These may be due to hormonal differences in the later trimesters or maybe coincidentally due to higher parasite levels in later pregnancy. Generally, malaria infections are associated with intrauterine growth restriction when they occur early in pregnancy and with preterm delivery when they occur later in pregnancy in areas with a high rate of malaria transmission.[18] There were no participants with severe parasitemia and this is because higher levels of parasitemia are usually accompanied by symptoms of malaria.

The findings of this study emphasize the need for the intermittent preventive therapy (IPT) and intermittent screening and treatment (IST) strategies among pregnant women in this environment to ensure control of malaria parasites in asymptomatic women. While the efficacy of Sulphadoxine-Pyrimethamine appears to be waning due to

parasite resistance, artemisinin based treatments appear to retain efficacy and safety particularly in later trimesters.[19]

This study has some limitations. The presence of submicroscopic parasitemia in participants who were negative by microscopy could not be ruled out as more sensitive detection techniques like polymerase chain reaction were not used. Submicroscopic parasitemia in non-pregnant persons has been put at 0.7 to 39.5%; in South America and 12 to 21% in Africa.[10] Further studies with larger sample sizes and exploring the effect of asymptomatic malaria parasitemia on fetal outcomes are required to corroborate the findings of this study.

In conclusion, pregnant women with asymptomatic parasitemia have higher levels of IFN-γ and TNF-α and may be at risk of fetal morbidity due to increased levels of cytokines released as a result of malaria parasite infection.

**Competing interests**

The authors declare that they have no competing interests.

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