BMC Immunology

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Markers of neutrophil activation and some immune and haematological indices in malaria infection during pregnancy

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Abstract

Background Neutrophils are the first responders to pathogen invasion and are important first-line defenders. The defence mechanism of activated neutrophils includes neutrophil extracellular traps (NETs) formation that immobilize pathogens, stop their spread within the tissues, and ultimately kill them. However, their roles in the context of malaria during pregnancy are still elusive. This study was conducted to investigate markers of neutrophil activation as well as immunological and haematological cellular responses during Plasmodium infection in pregnancy.

Method A total of 340 pregnant women aged between 19 and 42 years were recruited for this study carried out in South-east, Nigeria. All the subjects were tested for malaria parasite (MP) status. Those infected with human immunodeficiency virus (HIV) and those with any other co-morbidity were excluded from the study. A total of 45 (13.2%) of the 340 pregnant women were positive for malaria. To assess immune, haematologic and NETs markers in the MP positive group, 45 matched malaria-negative pregnant women from the malaria negative group served as controls. Thus, the final study population was grouped into two categories: 45 pregnant women infected with *Plasmodium falciparum* and 45 pregnant malaria-negative control group. The neutrophil elastase concentration, myeloperoxidase activity, total white blood cell counts, white blood cell differential counts, platelet counts and haematocrit were assessed via standard laboratory methods.

Results Findings from this study revealed lower levels of myeloperoxidase in the malaria- infected cohort ($p = 0.013$) than in the malaria negative cohort. The neutrophil elastase levels were also lower in the malaria negative group ($p = 0.042$). The total white blood cells, platelet and neutrophil counts were lower ($p = 0.046$, 0.012 and 0.015, respectively) in the malaria infected group when compared to the controls. Conversely, lymphocyte counts were higher in the malaria-infected group ($p = 0.003$). No cases with high parasitaemia were encountered among the pregnant women infected with *Plasmodium falciparum*.

Conclusion Malaria infection led to alterations in immune and haematological parameters in this group, with mild and moderate malaria parasitaemia in the study cohort. Although there were some significant differences, the assessed values remained mostly within the normal range. Further studies in a larger cohort assessing pregnant

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women infected with placental malaria and those with fatal outcomes are important to further investigate the role of NETs in malaria infection.

Keywords Immune responses, Neutrophil, Neutrophil extracellular traps, Malaria, Pregnancy

Introduction

Malaria remains a major public health concern. In 2022, there were an estimated 249 million cases of malaria in 85 malaria endemic countries and areas, translating to an increase of 5 million cases compared with 2021 [1]. Nigeria was one of the main countries contributing to this increase, with an additional 1.3 million cases. According to the World Health Organization (WHO), nearly half of the world's population is at risk of malaria. Africa, with an estimated 233 million cases in 2022, and Nigeria accounted for 94% and 27% of the global cases, respectively. Around 31% of malaria deaths worldwide occurred in Nigeria [1]. Malaria causes changes in infected individuals and these changes can vary according to malaria species, age, sex and other factors [2]. The scenario is further complicated by malaria infections during pregnancy. Recently, it has been estimated that globally, 121.9 million women reside in areas where they are likely to contract malaria during their pregnancies [3]. Pregnant women are particularly susceptible to malaria [4] and pregnancy-associated malaria results in substantial maternal, foetal and infant morbidity [5]. The pregnant women are particularly vulnerable to malaria because pregnancy reduces the immunity to the disease. This increases the risk of illness, severe anaemia, acute pulmonary oedema, renal failure, puerperal sepsis, postpartum haemorrhage, and death [6]. The susceptibility to malaria is attributed to immunological changes occurring during pregnancy, and to the placental tissue, which can serve as a reservoir for malaria parasites [7] and creates a new niche for the binding of infected erythrocytes during pregnancy [8]. *Plasmodium falciparum* parasites, which settle in the placenta, can cause severe illness and contribute significantly to maternal and infant mortality [9]. Environmental, parasitic and maternal factors also influence the severity of malaria during pregnancy [10]. Co-infections of malaria with other disease states in this population have been studied and their immunomodulatory effects have been documented [11, 12]. The risk and severity of certain infections are affected by pregnancy due to a combination of physiologic and immunologic changes [13]. Although there is little evidence that maternal immune system is globally suppressed during pregnancy, increased susceptibility to certain infections indicates important immunologic changes [14]. Over the past decade, significant progress has been made in reducing the global prevalence of malaria, particularly in Africa. However, pregnant women remain at high risk. There are reports that >50% of pregnant women harbour *P. falciparum* in malaria endemic regions [10]. Malaria during pregnancy contributes significantly to the estimated 5.5 million stillbirths and neonatal deaths that occur annually [15].

Neutrophils are the most abundant type of innate immune cells in humans and constitute the first line of defence against invading pathogens [16]. They migrate to infected peripheral tissues where they combat invading microorganisms. Defence strategies include phagocytosis, the release of reactive oxygen species (ROS) and the degranulation of anti-microbial peptides and immune mediators. Neutrophils digest microorganisms via the release of neutrophil elastase (a serine protease expressed in neutrophils), nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and myeloperoxidase (MPO) [17]. The latter is a peroxidase abundantly expressed in neutrophils. It is stored in the azurophilic granules of neutrophils and contains a heme-group, which is the cause of the green colour of secretions with a high content of neutrophils, such as pus and some forms of mucus.

Despite its anti-parasitic activity, neutrophil activation has been implicated in the pathogenesis of malaria. Activated neutrophils degranulate forming NETs that have been associated with malaria [18]. Therefore, we carried out this study to assess markers of neutrophil activation and immune and blood cell indices in pregnant women infected with malaria in a region mostly affected by *Plasmodium falciparum* malaria.

Materials and methods

Study participants

Pregnant women infected with *P. falciparum* and malaria negative pregnant women who served as controls were recruited for the study. Personal interviews/questionnaires were used to obtain demographic data. Signed informed consent was obtained from all participants. The study was conducted between April and December 2022.

Study design

The study had a cross-sectional design, and participants who met the inclusion criteria were recruited consecutively upon presentation to the ante-natal clinics.

Inclusion criteria

Pregnant women accessing ante-natal care at the selected hospitals were eligible to participate in the study.

Exclusion criteria

Pregnant women who were suffering from any other co-morbidities apart from malaria were excluded from the study.

Ethics statement

The study design was approved by the Institutional Review Board of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria (NAUTH/C5/66/vol.13/ver.III/90/2020/0590). and reported according to the STROBE checklist.

Study area

This study was carried out at Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, South-east, Nigeria. This is a tertiary healthcare facility that serves Nnewi, a commercial and industrial city and surrounding cities and towns in the state. Additional patients were recruited from Obioma Specialist Hospital, Nnewi and St. Charles Borromeo Specialist Hospital, Onitsha.

Laboratory analyses

Five millilitres of venous blood were drawn from each participant and dispensed into EDTA anticoagulated and plain (no anticoagulant) tubes. Anticoagulated blood was used for blood counts using standard automated methods. Serum samples from plain tubes were used for ELISA measurements of neutrophil elastase and myeloperoxidase.

Microscopy

Thick and thin blood films were prepared on glass slides. The dry thick and thin blood films were stained with Giemsa and Leishman stains respectively for malaria diagnosis and species identification. Two experienced microscopists independently examined the blood films. Malaria parasite density was determined on the basis of the number of parasites per 200 leucocytes on thick blood films. As previously described, the cut-off values for mild, moderate, and high parasitaemia were < 1000, 1000–5000, and > 5000 parasites/microlitre blood, respectively [19].

Blood counts

EDTA anticoagulated blood was used for full blood count and analysed using the Mindray BC-280 Haematology Autoanalyzer.

Neutrophil elastase (NE)

Elabscience™ (EL-H1946) was used to assess the human NE concentration in the serum according to the manufacturer's instructions. Briefly, the assay is based on sandwich-ELISAs. Micro-ELISA plates were pre-coated with an antibody specific for human neutrophil elastase. The optical density (OD) was measured via photometry at 450 nm. The OD value is proportional to the concentration of human neutrophil elastase. The detection limit was 0.78–50 ng/ml, with a sensitivity of 0.47 ng/ml.

Myeloperoxidase (MPO)

MPO is a peroxidase that is abundantly expressed in neutrophils, and MPO activity was assessed using the myeloperoxidase E-BC-K074 S, Elabscience™ colorimetric activity assay. The kit analyses were carried out according to the manufacturer's instructions. The detection limit was 16.95–3349 U/L and the sensitivity was 16.95 U/L.

Statistical analysis

GraphPad Prism 9.5.1 was used to analyse the data. The mean values, standard deviations, Kruskal-Wallis tests, Mann-Whitney tests, ANOVAs and Fisher's exact test were calculated. The level of significance was set at $P < 0.05$. The sample size was estimated on the basis of a malaria prevalence of 7.7% among pregnant women [20], resulting in a minimum sample size of 109.

Results

Malaria parasitaemia status and demographics

A total of 347 pregnant women consented, met the basic criteria and were recruited for the study. With screening and testing, 7 of them were excluded because of co-morbidities (hypertension-3; diabetes-2 and HIV positive-2). A total of 340 individuals met the inclusion criteria for participation. The flow chart (Fig. 1) shows the number of candidates enrolled and the subsequent classification. Forty-five (13.2%) of the 340 individuals who tested positive for malaria were included. A total of 45 gestational age matched malaria negative pregnant women from the participating cohort served as controls. Eleven of the 45 malaria infected women (24.4%) were primigravida while in the malaria negative group, 9(20%) were primigravidae.

The demographic characteristics of the study population are shown in Table 1. The mean age of the malaria positive pregnant women was 29.3 ± 4.7 years (range: 19–40), whereas the mean age of the malaria negative pregnant women was 30.2 ± 4.8 (range 21–39). There were no significant differences in the ages of the two groups ($p = 0.336$). There were no significant differences in parity, gravidity and socio-economic status (SES) between the study groups ($p = 0.732$, 0.800 and 0.518 respectively).

The haematological parameters of the MP positive group were compared with those of the control group (Table 2). There were significant differences in the total white blood cell ($p = 0.046$), neutrophil ($p = 0.015$), eosinophil ($p = 0.039$), platelet ($p = 0.012$) and lymphocyte ($p = 0.003$) count between the malaria positive pregnant women compared to the malaria negative controls. The

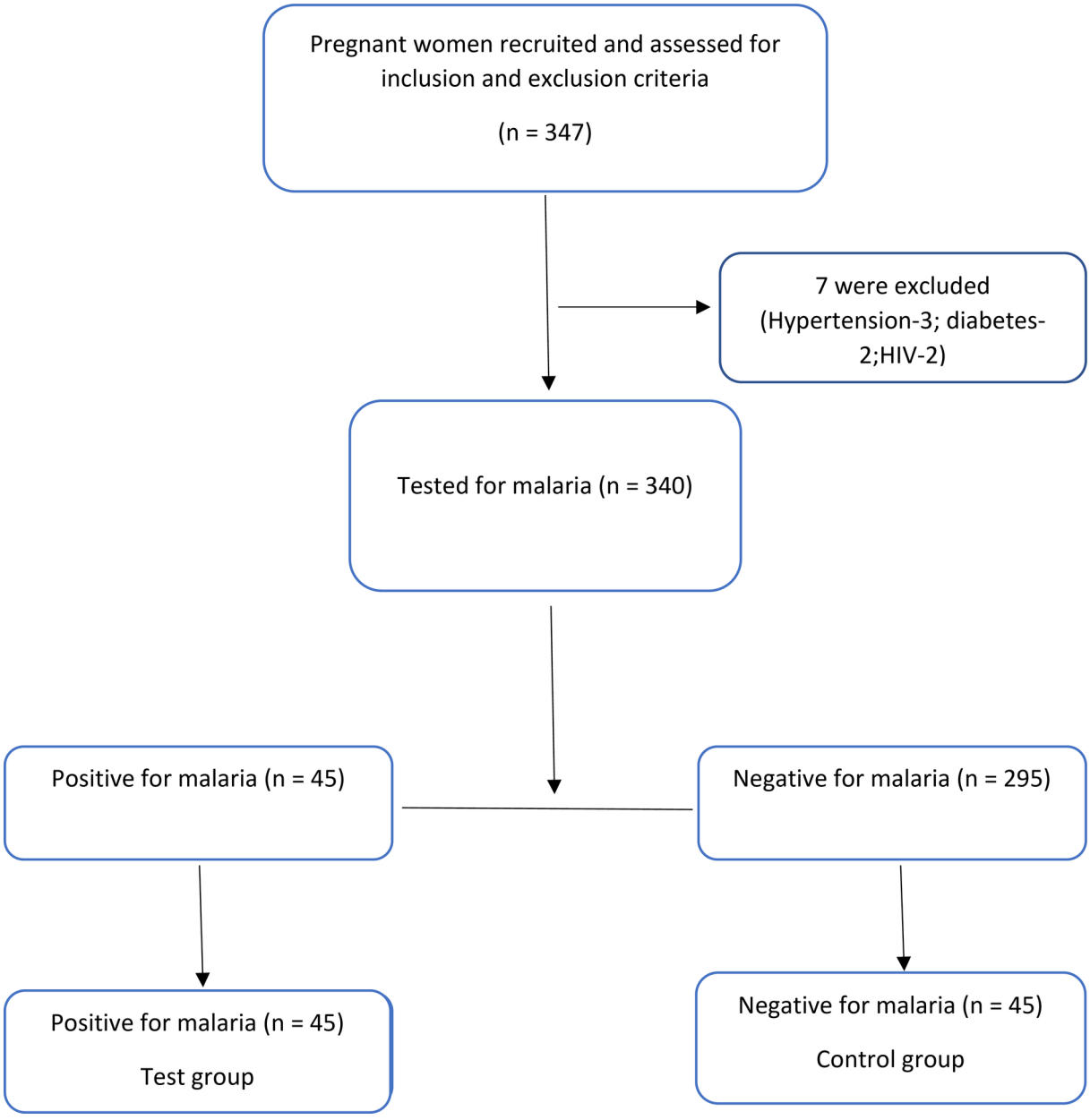

Fig. 1 Flow chart of the study participants

lymphocyte counts were higher in the malaria infected women, whereas the total white cell, neutrophil, platelet, and eosinophil counts were lower in malaria positive group. There were no significant differences in the haematocrit levels ($p = 0.373$), monocyte ($p = 0.713$) and basophil count ($p = 0.298$) among the study groups.

The malaria infected group was further categorized on the basis of the malaria parasite density. Twenty-one (46.7%) and 24 (53.3%) pregnant women had mild and moderate parasitaemia, respectively (Fig. 2 and Supplementary Table 1). There were no individuals with high parasitaemia. Based on normality test, either the t-test or Mann-Whitney and ANOVA or Kruskal-Wallis were used for comparison. When the blood cells of malaria negative group were compared with those of mild malaria group, significant differences were observed only in the eosinophil count ($p = 0.006$). However, when the malaria negative group was compared with the moderate malaria group, there were significantly higher total white blood cell counts ($p = 0.034$) and neutrophil ($p = 0.003$) in the malaria negative group compared to the moderate group. However, the lymphocyte counts were significantly higher in the moderate malaria group compared to the malaria negative group ($p < 0.001$). The neutrophil/lymphocyte ratio was significantly higher in the MP negative group ($p = 0.001$) compared to the moderate malaria

Table 1 Demographic characteristics of the study population

Variables	MP Positive *n* = 45	MP Negative *n* = 45	*P*-value
Mean Age (+ S.D.) (range in years)	29.3 ± 4.7 (19–40)	30.2 ± 4.8 (21–39)	0.336
Parity	2.24 ± 1.7	2.11 ± 1.8	0.732
Gravidity			
Primigravida	11(24.4%)	9(20%)	0.800
Multigravida	34(75.6%)	36(80%)	
Malaria parasite density			
Mild	21(46.7%)		
Moderate	24(53.3%)	-	-
High	0(0.0%)		
Socio-economic status (SES)			
Low SES	29(64.4%)	25(55.6%)	0.518
Mid-high SES	16(35.5%)	20(44.4%)	

Level of significance set at $p < 0.05$. Statistical significance was determined using Fisher's exact test

Table 2 Mean blood cell counts of the study population

Parameters	MP Positive *n* = 45	MP Negative *n* = 45	*P*-value
Haematocrit (%)	30.96 ± 2.68	30.40 ± 2.94	0.373
Total WBC ($x10^9$/L)	7.12 ± 1.96	7.91 ± 1.73	**0.046**
Neutrophil (%)	65.71 ± 7.46	69.27 ± 6.09	**0.015**
Eosinophil (%)	0.57 ± 0.54	0.86 ± 0.66	**0.039**
Lymphocyte (%)	30.98 ± 8.40	26.31 ± 5.97	**0.003**
Basophil (%)	1.22 ± 0.67	1.36 ± 0.53	0.298
Monocyte (%)	2.22 ± 0.87	2.13 ± 0.84	0.713
Platelet ($x10^9$/L)	206 ± 36	228 ± 47	**0.012**

Level of significance was set at $P < 0.05$. Significant values are in bold. Statistical significance was determined using t-test and Mann-Whitney test. The values for haematocrit, basophils and monocytes were not significantly different across the groups

group. The ANOVA revealed significant differences in the counts of neutrophils ($p = 0.018$). Using Kruskal-Wallis test, there were significant differences in lymphocytes ($p = 0.011$), and eosinophils ($p = 0.016$) and neutrophil/lymphocyte ratio ($P = 0.008$). Haematocrit, basophils, and monocytes did not differ in the intergroup comparisons based on malaria parasite density.

MPO activity was significantly higher in the malaria negative group ($p = 0.013$) between the malaria positive pregnant women and their malaria negative counterparts (Fig. 3). Similarly, NE levels were significantly higher in the malaria negative group compared to the malaria positive group ($p = 0.042$).

Intergroup comparisons revealed significant differences only in MPO activity and NE levels between the malaria negative group and the mild parasitaemia group, $p = 0.031$ and 0.046 respectively. There were no significant differences when comparing other groups (Fig. 4 and Supplementary Table 2).

Discussion

In Nigeria, studies conducted within the past three decades reported a prevalence of between 7.7% and 72% [20]. In the present study 13.2% of the pregnant women were positive for *Plasmodium falciparum*, the only malaria species detected in the studied population.

The clinical effects observed in pregnant women infected with malaria vary from asymptomatic [8, 10] to severe anaemia and death [10]. Women living in areas of low transmission display a lower degree of acquired immunity and experience more adverse effects than individuals living in high malaria transmission regions do. When the blood cell counts were assessed, the haematocrit did not significantly differ in the malaria infected population. Malaria is the major cause of anaemia in endemic areas. However, this depends on a number of other factors including infectious and nutritional status all of which contribute to anaemia [21]. A study of Congolese women reported that spatial and social factors, rather than malaria infection drive anaemia [22]. As observed in our study, pregnant women positive with malaria had mild to moderate malaria parasitaemia. This could explain why haematocrit values are similar in the malaria positive and malaria negative groups. However, the haematocrit values were lower than the reference range for women. This may be due to the physiologic plasma expansion observed during pregnancy, which was similar between the two groups.

Similarly, the total white blood cell (TWBC) counts were significantly different between the two groups ($p = 0.046$), with the malaria-infected individuals showing a tendency toward lower values. Leucocyte alterations are common during malaria infection, with generally lower levels in malaria infected patients than in healthy individuals [2, 23]. These reports are similar to our findings, whereas Elkhalifa and colleagues reported no significant difference in the TWBC [24]. However, leucocytosis is associated with clinical complications [2]. In this study, none of the malaria infected women had complicated or severe malaria.

There were significant differences in the number of neutrophils, eosinophils, and lymphocytes between the two groups. Increased neutrophil counts are a pathogenic factor in malaria [25]. Our findings revealed that the number of neutrophils was significantly lower in the pregnant women infected with malaria compared to the malaria negative group. In a study comparing neutrophil counts in malaria infected pregnant women, the authors reported findings similar to our results, with lower levels of peripheral blood leucocytes in malaria infected pregnant women than in their uninfected counterparts [26]. Similar to our findings, the values remained within normal ranges in the malaria infected group. This could be attributed to disease severity and some level of malaria

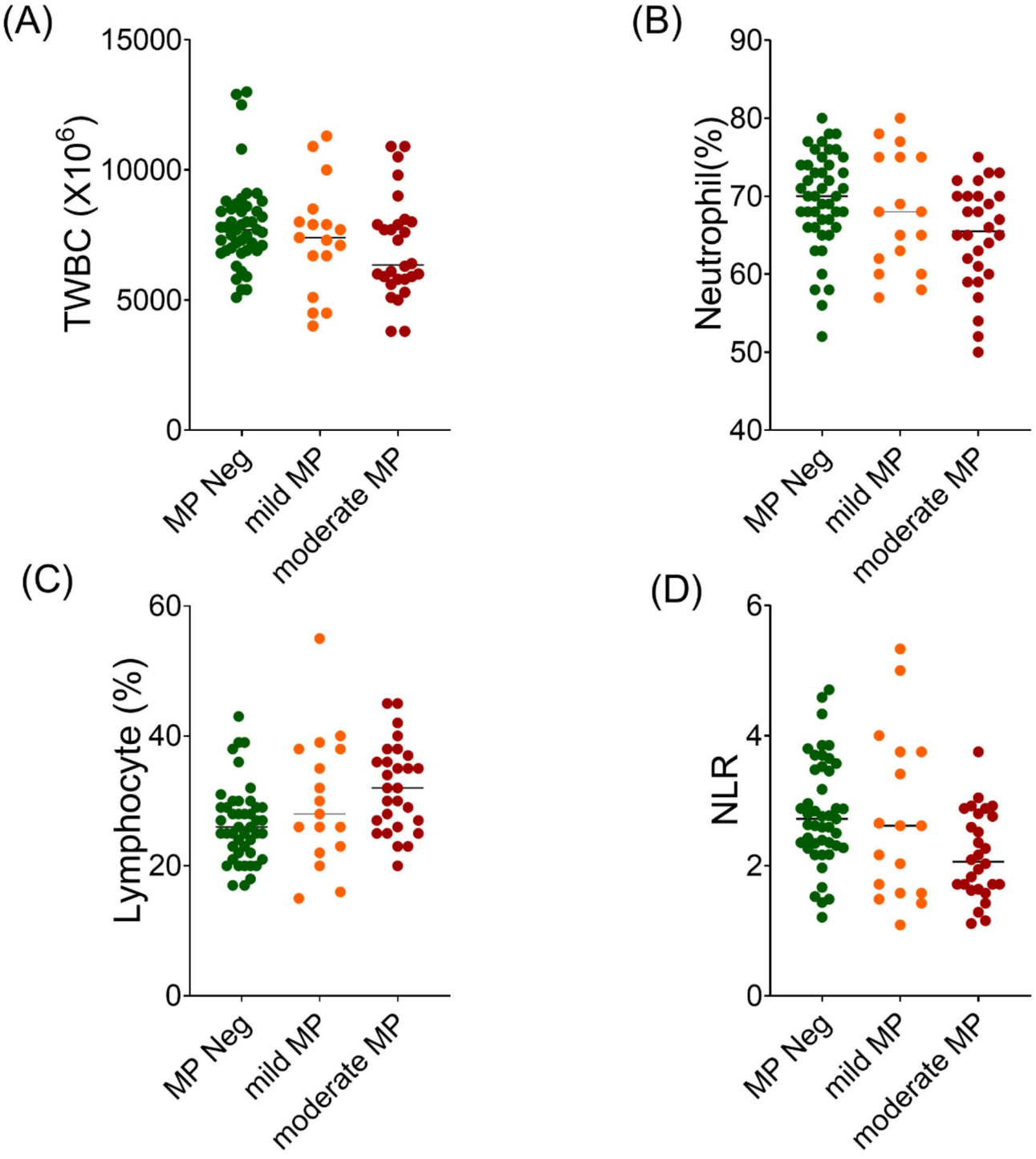

Fig. 2 Intergroup comparisons of immune cells in malaria negative pregnant control group (green) compared with the malaria infected population according to malaria density; orange (mild) and red (moderate) parasite density. Statistical significance was determined using Kruskall-Wallis test or ANOVA based on normality test. **A** Total white cell count **B** Neutrophil count **C** Lymphocyte **D** Neutrophil lymphocyte ratio. The *p* values can be found in Supplementary Table 1

immunity in the study population. Indeed, it has been reported that neutrophil counts are dependent on the study population and other associated factors, with increased numbers observed in infected children [27, 28]. Increased numbers were not observed in our study in the malaria infected pregnant women. It has been suggested that the observed decrease in peripheral neutrophil counts may partly reflect their accumulation of

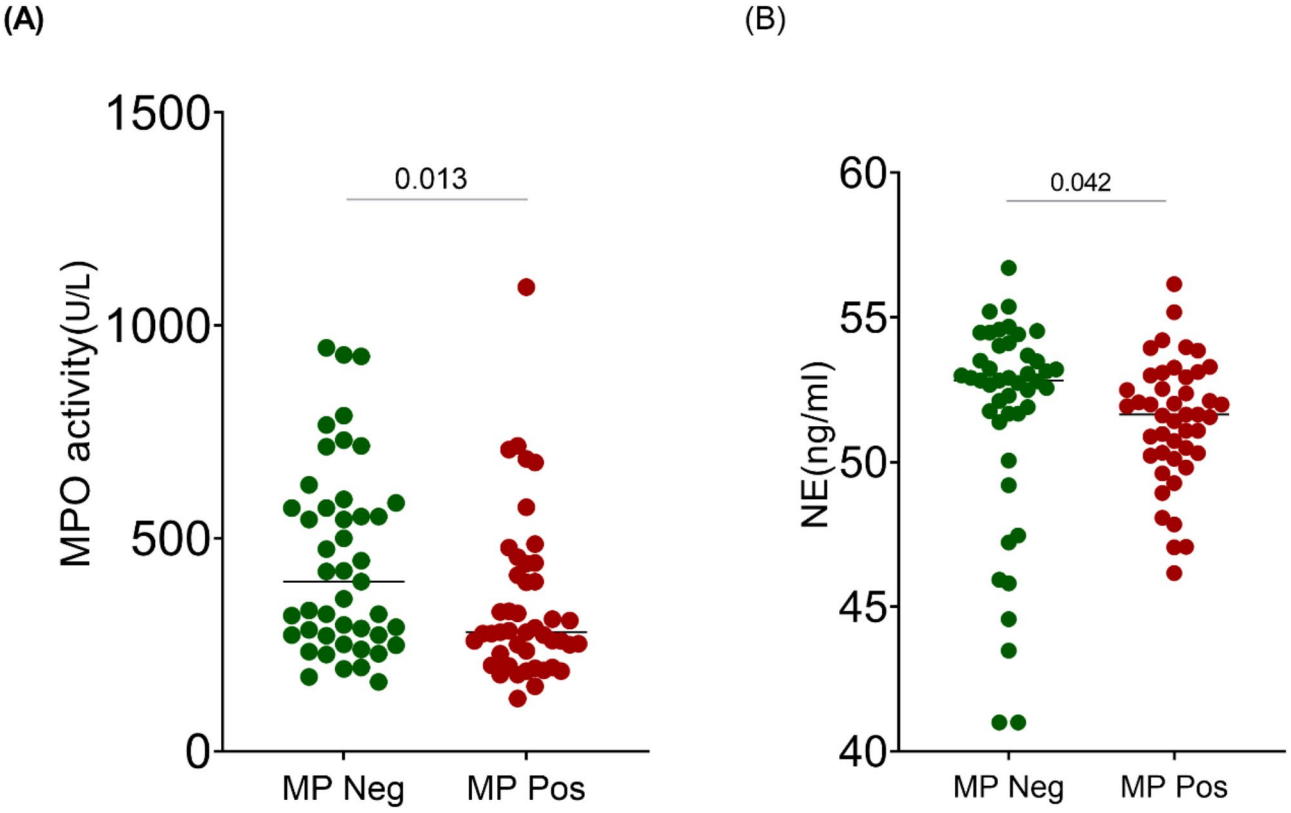

Fig. 3 Myeloperoxidase (MPO) activity ($p = 0.013$) and Neutrophil elastase ($p = 0.042$) in the plasma of malaria positive and malaria negative pregnant women. Statistical significance was determined using Mann-Whitney test. MP Pos: malaria positive; MP Neg: Malaria negative

neutrophils in the placenta as part of an immune infiltrate [26].

The higher lymphocyte counts observed in the pregnant women positive with malaria than in malaria negative group were interesting, as most studies reported lower lymphocyte counts in adults infected with malaria [24, 29]. The lymphocyte counts decrease during pregnancy through the 1st and 2nd trimesters and increases during the 3rd trimester in normal pregnancies [30]. Since the malaria negative pregnant group were gestational age-matched with the malaria positive group, the differences could be attributed to the malaria infection in this group.

Eosinophils play important roles in fighting parasitic, viral, fungal and bacterial infections [31]. Eosinophil counts were also significantly lower in the malaria infected group than in the uninfected group. A combined state of malaria infection and pregnancy could be responsible for this.

Platelet counts were significantly lower in the malaria infected pregnant women than in their uninfected counterparts. This finding is similar to those of previous reports in malaria infected individuals ranging from children to adults [10, 29, 32]. However, the platelet counts in the malaria infected pregnant women remained within normal ranges in the studied population.

Generally, the blood counts observed in the malaria infected individuals in this study were mostly within the normal range. Although malaria can play an important role in anaemia during pregnancy, anaemia during pregnancy is multifactorial with a relevant nutritional component [33] and malaria disease severity. Severe malaria is defined by clinical or laboratory evidence of vital organ dysfunction with hyper parasitaemia being a risk factor in P. falciparum [34]. None of the patients in the study population had hyper-parasitaemia. Statistical analysis revealed no significant differences in the haematological parameters studied in the women with mild or moderate malaria parasitaemia. Given the level of malaria transmission in the study region, the findings of mostly mild and moderate parasitaemia and some sub-clinical malaria infections among the pregnant women, there is most likely some level of immunity in the population. It has been reported that adults who live in malaria endemic regions acquire some immunity to malaria infection with repeated parasite infections [35, 36]. Despite this acquired partial immunity, pregnancy associated malaria is relatively common but is not always associated with pathology [4]. Pregnant women

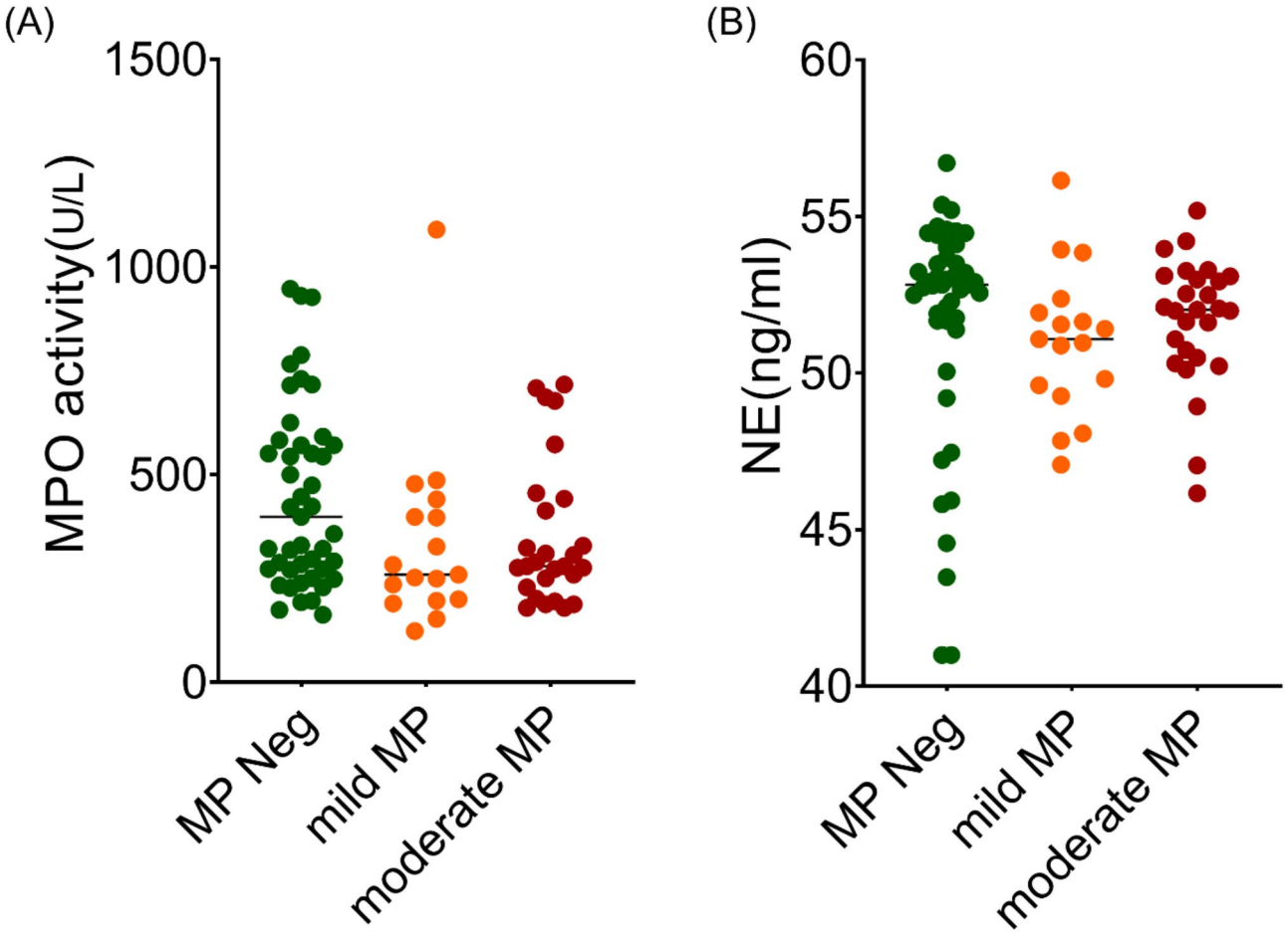

Fig. 4 Intergroup comparisons of myeloperoxidase activity and neutrophil elastase in malaria negative pregnant control group (green) compared to the malaria infected population according to malaria density; orange (mild) and red (moderate) parasite density. Statistical significance was determined using Mann-Whitney test. The *p* values are shown in Supplementary Table 2

living in areas of high transmission sometimes experience asymptomatic peripheral parasitaemia [37]. In this study, some of the women who were smear positive for malaria reported no symptoms ($n = 2$), which could be attributed to some level of immunity. This would, of course, impact the haematological findings. The finding of mostly mild and moderate malaria is not unexpected, as this is an endemic region where most adults have developed a certain level of immunity. In a study conducted in the Bamenda region of Cameroon, a country that shares borders with Nigeria, with similar malaria transmission, 74.4% of the malaria positive patients in the study had mild malaria parasitaemia [29]. In our study, 46.7% of the pregnant women had mild malaria, while 53.3% had moderate malaria parasitaemia. The difference could be attributed to the state of pregnancy. Overall, immune status, co-infection, age and parasite burden affect haematological parameters as shown in a study in a similar population [10].

Neutrophils play important roles as effector cells in the immune defence against malaria [38]. However, the role of neutrophils in malaria remains elusive [26]. Clinical protection from *Plasmodium falciparum* correlated with a neutrophil respiratory burst, suggesting a role in parasite clearance [39]. Indeed, neutrophils play a role in the clearance of malaria parasites via phagocytosis, the production of reactive oxygen species (ROS) and possibly NETs formation [18]. Importantly, it has been suggested that neutrophils are double edge swords that also play a role in the pathology of malaria [38]. Neutrophil elastase is a serine protease expressed in neutrophils, and its level and activity are indicators of disease state and severity [40]. Plasma concentrations of neutrophil elastase were assessed in this study and there were significantly higher levels in the malaria negative pregnant women compared to the malaria infected group (Fig. 3). This was unexpected as malaria infection should lead to increase in inflammation in the test group compared with the control group.

Myeloperoxidase (MPO) is a leucocyte-derived enzyme that is mainly secreted by activated neutrophils [41]. Unexpectedly, MPO activity was significantly lower in

malaria infected pregnant women compared to their uninfected counterparts. Although neutrophil counts are lower in the malaria infected group, and this may be due to sequestration or consumption during immune defence, it was expected that the MPO activity would have been greater in this group. However, this was not the case from in our findings.

During infections and inflammatory diseases, MPO is increasingly released from azurophilic granules of activated neutrophils into phagocytic vacuoles and extracellular spaces [42]. This was not observed in the case of malaria infected pregnant women compared with their uninfected counterparts. During pregnancy, major adaptations occur in the maternal immune system to protect the mother and the foetus from pathogens while avoiding detrimental immune responses against the allogeneic foetus [13]. Interestingly, in a study on an animal model of malaria (*P. yoelii*), and in MPO knock-out mice, it was suggested that MPO modulates the adaptive immune response during malaria infection, leading to attenuated parasite clearance [41]. We infer that there could be a possible protective adaptive mechanism in place in these malaria infected pregnant women in relation to the MPO to increase parasite clearance.

However, when comparing these results further based on malaria parasite density, a trend was observed for both MPO activity and NE. it is interesting to observe that in mild parasitaemia, MPO and NE levels are lowest leading to significant differences with the malaria negative group. When the parasitaemia levels are higher as seen with the moderate parasitaemia group, both MPO and NE levels are higher and were not significantly different from the malaria negative group. This would then suggest that increased disease severity may lead to higher levels of MPO and NE, in line with the increased inflammation that is a hallmark in hyper-parasitaemia [43]. Therefore, assessing the effect of increased malaria disease burden could shed more light on this finding.

A limitation of this study is that the NE enzyme concentration was assessed, while the enzyme activity could have provided a better picture of the neutrophil elastase response to malaria.

Another limitation of the study was that no pregnant women with high parasitaemia were encountered during recruitment for this study. This could be due to health interventions such as use of intermittent preventive treatment in pregnancy (IPTp) for malaria in the 3 study recruitment sites. Information on IPTp use in the studied population would have been useful, however this data was not obtained.

Conclusion

The findings from this study show that malaria infection led to alterations in immune and haematological parameters in this group, with mild and moderate malaria parasitaemia in the study cohort. It is important to note that though there were some significant differences, the values remained mostly within the normal range. Further studies in a larger cohort assessing pregnant women infected with placental malaria and those with severe disease or fatal outcomes are important to further investigate the role of NETs in malaria infection.

Abbreviations

HIV	Human Immunodeficiency syndrome
MP	Malaria parasite
NETs	Neutrophil extracellular traps
ROS	Reactive oxygen species
MPO	Myeloperoxidase
NE	Neutrophil elastase
SES	Socio-economic status
WBC	White blood cell counts
TWBC	Total white blood cell counts

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12865-025-00709-4.

Supplementary Material 1.

Acknowledgements

The authors are grateful to the study participants and staff of the hospitals who assisted with participant recruitment.

Authors' contributions

Conceptualization RC and MH; Drafting the manuscript: RC, CA; Patient evaluation and recruitment GU, CA; Performing laboratory analyses CA, AE, DA, RC. Evaluating and analysing data: CA, RC, MH. Finalizing the manuscript RC., CA, AE, DE, GU, MH.

Funding

The authors received no financial support for this study.

Data availability

The research data is available on request.

Declarations

Ethics approval and consent to participate

The experimental protocol and study design was approved by the institutional review Board of Nnamdi Azikiwe University Teaching Hospital (NAUTH/C5/66/vol.13/ver.III/90/2020/0590).
Informed consent was obtained from all study participants. This study was conducted in accordance with the Helsinki declaration.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Received: 26 September 2024 / Accepted: 1 April 2025
Published online: 08 April 2025

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