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Relationship between Neutrophil-lymphocyte Ratio, Monocyte-Lymphocyte Ratio and Parasitaemia in Determining the Severity of Malaria Infection in Children

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ABSTRACT

Background: Malaria often presents as a febrile illness which causes noticeable changes in the haematological parameters. There were inconsistencies with the report on the relationship between the haematological parameters and level of parasitaemia. **Objectives**: This study therefore aimed to ascertain the correlation between the level of parasites in the blood, the neutrophil-lymphocyte ratio (NLR) and the monocyte –lymphocyte ratio (MLR) as a measure of severity of malaria infection. **Materials and Methods:** This was a cross-sectional study involving three groups of children: complicated malaria, uncomplicated malaria and healthy controls. Haematological profile was done using a haematology auto-analyzer. Parasite density was done using microscopy. The study's data was analyzed utilizing the Statistical product and services solutions (SPSS) version 25. **Results:** The mean level of NLR and MLR was higher in children with complicated than uncomplicated malaria ($p = 0.023$); and normal healthy children ($p < 0.001$). About half 46.3% of the children in the complicated group had parasitaemia in the range of >100-5000 parasites /µL while 47.5% of children in the uncomplicated group had parasitaemia in the range of 500-1000 parasites /µL. The mean parasite density for complicated and uncomplicated groups were 4542.9 and 1666.6/ µL respectively. A positive significant correlation was seen between NLR, MLR and the parasite density in the complicated malaria group {r= 0.623, P=0.022)**. Conclusion**: The NLR and MLR correlate positively with the level of parasitaemia. Consequently, these ratios could serve as a surrogate for the level of parasitaemia and hence the severity of malaria in children.

Keywords: Complicated malaria, Malaria, Neutrophil-lymphocyte ratio, Monocyte lymphocyte ratio, parasitaemia,

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INTRODUCTION

Malaria still remains a disease of public health concern for over a third of the world's population.[1] Malaria infection is caused by a mosquito borne parasite called *Plasmodium spp*. Malaria is the primary cause of mortality, hospital admissions, and outpatient visits for most individuals living in malaria endemic regions, especially for children under the age of five. [2,3]

Two of the five parasite species that can cause malaria in humans are *Plasmodium falciparum* and *Plasmodium vivax*, and they are the deadliest. [4] *Plasmodium falciparum* infections account for the great majority of malaria-related deaths; *P. vivax* infections can result in severe febrile illness. [5] In addition to developing mechanisms to reduce the inflammatory response to the parasite that causes the acute fever symptoms after recurrent infection, people living in malaria-endemic areas gradually evolve systems to either eradicate parasites or suppress their multiplication. [6] Children are therefore more likely than adults to have clinical malaria, serious illness, or even death because they have not yet developed immune defenses. [7,8] Additionally, it has been discovered that visitors to areas where malaria is endemic have severe cases of the disease. Severe or complicated malaria is characterized by increased parasitaemia of greater than 5% and/or organ failure signs. These symptoms include hypoglyceamia, unconsciousness, cerebral malaria, end stage renal disease, and prostration. [9]

It has been established that malaria, which is a major cause of death in endemic nations, has both direct and indirect impacts on the haematological parameters. Haematological alterations are the most frequent side effects since malaria parasites are blood parasites. [10] They thus represent some of the key players in the pathogenesis of malaria. [11] Malaria infections are most frequently accompanied by haematological alterations, and these changes are crucial to the pathophysiology of malaria. Major cell lines, such as red blood cells, leukocytes, and thrombocytes, are impacted by these modifications. [12] Monocytes tend to become phagocytic in the presence of protozoa, bacteria, or fungal infections. A study by Antwi-Baffour *et al*, found a significant rise in monocyte count in people with malaria infections. [13] Additionally, lymphocytopenia brought on by malaria infections is accompanied by an increase in neutrophil count, which is typically interpreted as an indication of systemic inflammation.[13]

Haematological abnormalities that are seen in severe malaria infection may include: anaemia, thrombocytopenia, splenomegaly, leucopenia, leukocytosis, mild-to-moderate atypical lymphocytosis, and less frequently, disseminated intravascular coagulation. [10,14] In addition to anaemia, individuals with complicated malaria had more severe neutropenia, lymphocytosis, monocytosis, and thrombocytopenia than patients with uncomplicated malaria .[15]

According to clinical evidence, patients with acute, uncomplicated malaria have a high level of circulating neutrophils in contrast to circulating lymphocytes, which fall during *P. falciparum* infections.[16] Cytokines are released during *Plasmodium* infections and may stimulate circulating neutrophils. [17] Neutrophils that have been activated are armed with a variety of tools to establish an immunological defense against the parasite. On the other hand, although the underlying mechanism is still unknown, these neutrophils may possibly play a role in the pathogenesis of severe malaria.[16] Likewise, Warimwe *et al* reported that children with asymptomatic *P. falciparum* infection had a high monocyte to lymphocyte ratio in clinical malaria throughout follow-up in Kenya.[18] This is due to the fact that with repeated natural exposure to malaria infection, a person may not have symptoms but may instead harbor the parasite, which will continually stimulate their immune system and produce an increase in the number of monocytes.[19] Therefore, older children and adults continue to be vulnerable to asymptomatic *Plasmodium* infections, which are frequently brought on by *P. falciparum*, to which immunity is likely to never develop. [20] This study therefore

aimed to determine if any correlation exists between the NLR and MLR in children with malaria and the level of parasitaemia. This may aid better treatment of malaria and thus reduce mortality from complicated cases.

MATERIALS AND METHODS

Study design

This was a cross- sectional analytical study carried out from December 2022 to August 2023.

Study Setting

This study was carried out in the paediatrics' wards and clinics of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State, Nigeria. The University of Nigeria Teaching hospital (UNTH) Enugu is a Federal tertiary health care institution that provides a wide range of medical, surgical, diagnostic, out- patient, in-patient, rehabilitative, and support services to its catchment population, which includes all the states in the South Eastern part of Nigeria and other neighbouring states. Enugu state population comprises mainly civil servants, health care workers, students, and traders.

Sample size calculation

The minimum sample size was obtained using the understated formula according to Naing, Win and Rush. [21]

$$N = Z^2 x\, P\,(1-P)/d^2$$

Where; N= Minimum sample size, Z = confidence interval (1.96), d= desired level of significance (0.05), P = Prevalence rate (prevalence rate of complicated malaria is 5.5%). [22] This gave a total sample size of 80 for each arm of the study.

Study Participants

The patients for this study were children 6 months to 12 years old admitted into the paediatric ward of UNTH Enugu, from out-patient paediatric clinics which hold daily at the UNTH Enugu and also from the children emergency ward. The healthy controls were made up of children that came for immunization at the hospital and also those that came for pre-school screening exercises. There were three groups of participants: the first group were children with complicated malaria, the second group were children with uncomplicated malaria, and the third group were children who did not have malaria.

Inclusion criteria:

Children who were 6 months – 12 years old with an axillary temperature ≥37.4°C or with a history of fever within the last 72 hours.

Exclusion criteria:

Those children above 12 years of age and below 6 months of age were excluded. Children diagnosed with other disease conditions such as bronchopneumonia or meningitis etc and children who have received a full course of artemisinin combination therapy (ACT) in the current illness or those that were on malarial prophylaxis were excluded.

Ethical approval and informed consent

Ethical approval was obtained from the Institutional Review Board of University of Nigeria Teaching Hospital, Ituku/Ozalla (application reference no: NHREC/05/01/2008B-FWA00002458-IRB00002323 – UNTH/HREC/2023/06/530). A written informed consent was also obtained from each participant's mother or caregiver; and assent obtained from older children before enrollment into the study.

Sample collection

Two millilitres of venous blood were collected from the children by venesection into an ethylene diamine tetraacetic acid (EDTA) anticoagulant tube and transported to the haematology laboratory. Peripheral thin blood smears were done and stained with Leishman for morphology. Thick and thin blood smears were made and stained with Giemsa and inspected for plasmodium parasites by two experienced independent microscopists as a way of guaranteeing appropriate quality control.

Full Blood Count Estimation

The full blood count (FBC) was done using the automated analyzer – Mindray BC-880 haematology autoanalyzer based on the principle of impedance counting. Whole blood was collected in RDTA bottle was also used for FBC.The FBC (haemoglobin estimation, packed cell volume and red cell indices, total white cell count with differentials and platelet count) were done immediately after sample collection. The immune cell ratios – NLR and MLR were calculated from the results of FBC using the formulae below:

NLR = neutrophil count x 10^9/L /lymphocytes count x 10^9/L

MLR = monocyte count x 10^9/L/lymphocytes count 10^9/L [23]

Parasitological analysis

The smears were air dried and afterwards the thin smears were fixed using absolute methanol. The staining of the slides was done using 10% Giemsa stain solution. The slides for staining blood films was placed face up on a staining rack. The Giemsa stain was poured slowly on the slide until the blood films were covered completely. The timer was set to 40 minutes for the staining. The stain from the slides was flushed by dropping clean water over them. The slides were allowed to air-dry and then viewed under the microscope.[24]

Estimation of malaria parasite density: Plasmodium parasites were counted against 200 WBC in thick film. Five hundred WBCs were counted where less than 9 parasites were observed. Counting was done in the thin film against 2000 red blood cells. When counting was completed, the parasite density was calculated on the basis of the patient's actual white cell count.

$$\text{Parasites / } \mu\text{L blood} = \frac{\text{Number of parasites counted} \times \text{Actual white blood cells Count}}{\text{No. of white blood cells counted}}$$ [25]

Data analysis

Data was reported as mean±standard deviation, appropriately. Variables were compared between the clinical subgroups (complicated, uncomplicated, and healthy controls) by using the non-parametric measures. Differences between subgroups were analyzed for statistical significance using the Analysis of variance test. Possible correlations between levels of cytokines and parasite density were identified using Pearson's correlation coefficient. All statistical tests were done using SPSS version 21.0 for Windows (SPSS Inc., Chicago IL, USA). *P* values below 0.05 were considered statistically significant.

RESULTS

General characteristics

The overall mean age of the participants was 7.3 ±3.4 years (range 6 months - 12 years). Most of the participants, 237 (98.8%) were of the Igbo tribe and the male-female ratio was 1:1. The age and gender distribution among the three groups were similar (P > 0.05). Other characteristics of the three groups including tribe, and clinical signs were as shown in Table 1.

Level of parasitaemia

About half (46.3%) of the children in the complicated group had parasitaemia in the range of >100-5000 parasites /µL while 47.5% of children in the uncomplicated group had parasitaemia in the range of 500-1000 parasites /µL. No parasites were seen in the control group. The mean parasite density for complicated group was 4542.9 and 1666.6/ µL for the uncomplicated group. Further details about parasitaemia were as shown in Figure 1.

Haematological parameters among the groups

A significant difference was found between these haematological parameters, haemoglobin level, total white cell count, monocyte count and neutrophil count (*P* value <0.001). This is shown in Table 2.

Table 1: Characteristics of the children who participated in the study

Variable	subgroup	Group A (N-80)	Group B (N-80)	Control (N-80)	F*	p-value
Age (Years)	Mean age	6.85±3.65	7.63±3.36	7.45±3.28	0.83*	0.44
	<1	11(13.7%)	8(10%)	4(5%)	5.57[a]	0.23
	1-5	13(16.3%)	9(11.2%)	16(20%)		
	6-12	56(70%)	63(78.8%)	60(75%)		
Ethnic group	Igbo	78(97.5%)	79(98.8%)	80(100%)	-	
	Others	2(2.5%)	1(1.2%)	0		
Religion	Christianity	76(95.0%)	77(96.2%)	78(97.5%)	0.69[a]	0.71
	Islam	4(5.0%)	3(3.8%)	2(2.5%)		

*ANOVA (analysis of variance); [a] = Chi square

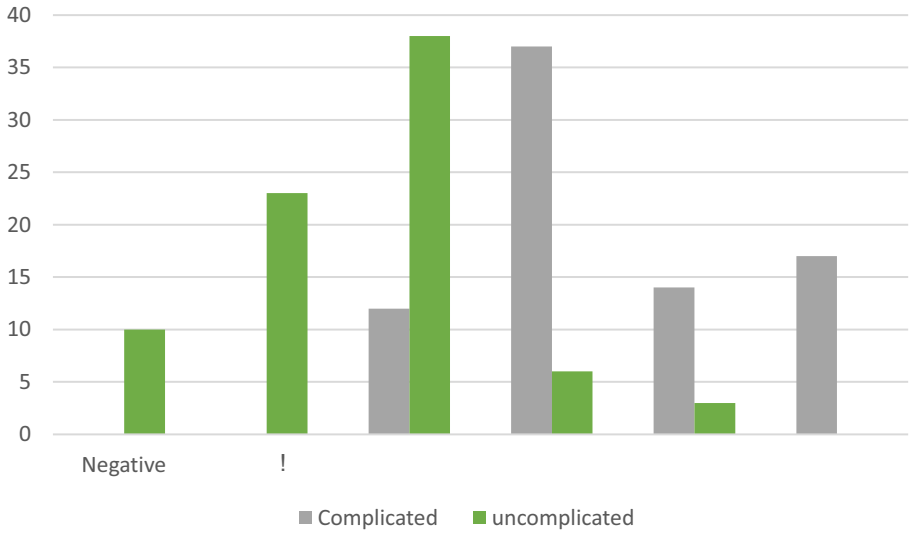

Figure 1: Parasite densities in complicated and uncomplicated groups

Table 2: Mean values of the haematological parameters of the children in various groups

Haematological parameters	Complicated	uncomplicated	Control	P value*
Haemoglobin (g/dl)	7.8±1.6	10.1±1.3	11.7±1.8	< 0.001
TWBC ($x10^9$/L)	11.2±4.8	8.1±4.4	5.6±1.87	<0.001
Neutrophil ($x10^9$/L)	7.5±3.9	5.4±3.5	2.9±1.4	<0.001
Monocytes ($x10^9$/L)	0.3±0.2	0.2±0.1	0.1±0.1	< 0.001
Lymphocyte ($x10^9$/L)	3.4±2.4	2.6±1.3	2.5±1.2	0.016
Eosinophil	0.1±0.1	0.1±0.1	0.1±0.1	1.00

*TWBC- Total White cell count; p is significant at 0.05, * -ANOVA*

Table 3: Mean levels of Neutrophil lymphocyte ratio and Monocyte lymphocyte ratio in the 3 groups

Study group	NLR	MLR
Complicated		
Mean	3.69	0.13
StDev	3.99	0.18
Max	22.59	1.2
Min	0.44	0
Uncomplicated		
Mean	2.38	0.08
SD	1.53	0.10
Max	7.17	0.67
Min	0.48	0
Control		
Mean	1.29	0.06
SD	0.70	0.04
Max	3.26	0.15
Min	0.26	0
P value	<0.001	<0.001

NLR - Neutrophil lymphocyte ration; MLR - Monocyte lymphocyte ratio; P - < 0.05

Table 4: Correlation of parasite density with NLR and MLR in the three groupsControl parameter correlation Complicated Uncomplicated

Parasite density vs NLR	**r**	0.923	0.523	-0.049
	P-value	0.0001	0.348	0.69
Parasite density vs MLR	**r**	0.884	0.687	-0.131
	P-value	0.001		0.615

r – Pearson's correlation coefficient; NLR - Neutrophil lymphocyte ration; MLR Monocyte lymphocyte ratio; P - < 0.05

The neutrophil - lymphocyte ratio and Monocyte - lymphocyte ratio in children with complicated and uncomplicated malaria, and normal healthy children

The mean levels of NLR in the complicated, uncomplicated and the healthy groups were 3.7±4.0, 2.4±1.5 and 1.3±0.7. The difference between the three groups were significant *P*<0.001). However, there was no significant difference between the levels in uncomplicated and normal healthy children (= 0.14^{P}). On the other hand, the mean levels of the MLR in the complicated, uncomplicated and healthy groups were 0.1±0.7, 0.08±0.1 and 0.06±0.0 respectively. The mean levels of MLR did not show any significant difference in the three groups (*P* = 0.06); and in between the three groups (*P*> 0.05). Details are as shown in Table 3.

Correlation between immune cell ratios and parasite density in the three groups

A positive significant correlation was seen between NLR and the parasite density in the complicated malaria group {r= 0.923, p=0.0001). The relationship between NLR, MLR and parasite density in the uncomplicated and control groups was as shown in Table 4.

DISCUSSION

We found a higher number of parasites per microliter in the complicated group. About half of the children studied had peripheral blood parasitaemia in the range of 100 to 5000 parasites per microliter. This finding is expected since parasite density has a direct relationship with malaria severity. Patients with complicated malaria often have high numbers of parasites in the blood and in some organs, such as the liver depending on the stage of development of the parasites. The presence of these parasites often evokes an immune reaction in order to get rid of the invading organisms. [26]

The innate immune system is the first line of defense that orchestrates the removal and destruction of parasites. Several haematological parameters are often elevated or depressed during the malaria infection. Neutrophils and monocytes are innate immune cells responsible for the host's defense against malaria. Activated monocytes use phagocytosis, cytokine synthesis, and antigen presentation to help lower the parasite burden. Nevertheless, monocytes have also been linked to the pathophysiology of severe malarial infection by releasing harmful inflammatory cytokines that cause vascular dysfunction and systemic inflammation.[27] Some of these cytokines include interferon gamma, tumour necrosis factor and other interleukins. We found a higher absolute number of neutrophils and monocytes in the complicated group than in the uncomplicated and healthy controls. Therefore, it may be assumed that the frequency of neutrophils and monocytes in peripheral blood would reflect the condition of the person's immune response to the illness. Thus, circulating monocytes

and neutrophils may function as a proxy for malaria parasitaemia.[13]

The etiology and consequences of malarial disease can be attributed in part to haematopathological abnormalities. The significant phagocytosis and destruction of the parasitized erythrocytes seen with increasing severity may be a contributing factor for the lower mean level of haemoglobin concentration reported in children with complicated malaria.[28] Reduced immunity and septicemia, which induce leukocytosis with the goal of battling sepsis, may be the cause of the noticeably increased amount of total white blood cells in complicated malaria.[29,30] Several investigations conducted by Atwi-Bafour, Maina, and others support this conclusion.[13,30, 31]

In a group of malaria patients, Kotepiu *et al* discovered decreased levels of haemoglobin, Total white cell count, neutrophils, mononcytes, and lymphocytes while Lui *et al* also found a lower level of TWBC.[32,33] The reason they propounded for a lower level of WBC was that of leucocytes being shifted to the margination pool and thus absent from the peripheral circulation. It is therefore not surprising to find a significantly higher NLR in children with complicated malaria as observed in this study. There was a significant correlation between most immune-inflammatory markers and malaria parasite density in infected patients. A positive correlation was seen between NLR and the parasite density and this relationship was noted to be statistically significan There was also a positive correlation between MLR and parasite density {r=0.884, p=0.001). This is similar to the findings of Idemudia *et al,* (2021).[34] The monocyte to lymphocyte ratio obtained correlated positively with the presence of malaria as well as the level of parasitaemia. [13]

The NLR was proposed as a marker for stress and inflammation. Therefore, the higher the levels of malaria parasitemia, the higher the induced stress and inflammation level. Malaria, being a highly inflammatory condition, requires intricate interactions between the host, parasite, and environmental factors underlying its pathogenesis. [35] The human response to malaria involves a variety of actions, including both cell-intrinsic and systemic pathways, but in a naïve host, non-specific reactions dominate the early responses. However, some studies reported a significant negative correlation between MLR with malaria parasitemia which is not in tandem with our findings. [37]

Strengths and Limitations

This study compared three different groups: those with complicated malaria, uncomplicated malaria and the healthy malaria groups.

This was a single center study with a limited sample size. A multi center study with a larger sample size may be required.

CONCLUSION

The NLR and MLR correlate positively with the level of parasitaemia. Consequently, these ratios could serve as a surrogate for the level of parasitaemia and hence the severity of malaria in children.

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Author contributions

AOU, RCC conceptualized and designed the study. AOU, RCC, FAE, EOU and PCE contributed to implementation of the project and revision of the manuscript. All authors were involved in the writing and revision of the manuscript. The authors read, approved the final manuscript and agree to be accountable for all aspects of the work.

Data availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

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Conflicts of interest
None to declare

Ethical approval:
The study was approved by the Institutional Ethics Committee.

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