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MALARIA PARASITE DENSITY, HAEMATOLOGY AND NUTRITIONAL INDICATORS IN BREASTFEEDING CHILDREN 0-2 YEARS AT CHUKWUEMEKA ODUMEGWU OJUKWU UNIVERSITY TEACHING HOSPITAL AWKA: A CROSS-SECTIONAL STUDY

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ABSTRACT

Malaria remains a major health issue caused by infected Anopheles mosquito especially in tropical and subtropical regions. Knowledge of haematological profile of infected persons is key in accurate diagnosis and effective treatment. This survey investigated the malaria parasite density, haematology and nutritional indicators in breastfeeding children 0-2 years at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka. This hospital-based cross-sectional study was conducted from June to August 2024. Venous Blood samples were collected from the patients and analysed using microscopy and Automated Haematology Analyzer. Height and weight were measured using meter rule and weighing balance. Information on their breastfeeding practices was obtained with the aid of a questionnaire. Of the 114 breastfeeding children sampled, only 32.5% (n=37) were exclusively given breast milk. Those who received breast milk and water were 15.8% (n=18) while those who received breast milk and other food were 51.8% (n=59). Results from the survey showed that 26.3% of the breastfeeding children were positive for malaria. Male children had a higher mean parasite density than their female counterparts but the difference was insignificant ($p>0.05$). Parasite density across age groups increased as age increased, however, the variation in parasite density was not significantly different ($p>0.05$). A significant negative correlation was found between the height of breastfeeding children and MCV ($r=-0.39$, $p<0.01$) as well as MCH ($r=-0.22$, $p<0.05$). Additionally, the weight of the children displayed a significant negative association with PCV ($r=0.19$, $p<0.05$), MCV ($r=-0.34$, $p<0.01$), and EOS_PLA ($r=-0.19$, $p<0.05$). Regular screening and monitoring are recommended. Implementing preventive measures such as insecticide-treated bed nets and antimalarial prophylaxis can help reduce the risk of malaria transmission.

Keywords: Breastfeeding, Children, Haematology, Malaria, Nutrition

INTRODUCTION

Malaria is a life-threatening disease caused by parasites transmitted to people through the bites of infected female Anopheles mosquitoes. Of the more than 120 *Plasmodium* species known to exist, only five cause malarial infections in humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae* and Plas*modium knowlesi* (Emmanuel *et al*., 2024). *P. falciparum* accounts for an estimated 60% of outpatient hospital visits in Nigeria, 30% of hospitalizations, 30% of under-five mortalities and 25% of infant mortalities (Noland *et al*., 2014). Those at greatest risk of severe forms of the disease, and death, are children under the age of 5 years, and pregnant women (WHO, 2022b). Malaria infections occur in five WHO regions and globally an estimated 3.4 billion people in 91 countries and territories are at risk of being infected with malaria and developing the disease with an additional 1.1 billion at high risk (WHO, 2018). In 2021, there were an estimated 247 million cases of malaria and 619,000 deaths worldwide (WHO, 2022a) Malaria is mainly endemic in tropical regions and developing countries due to the poor sanitation conditions in these developing countries (Bloland *et al*, 2002). As the parasite develops in the erythrocyte, numerous waste substances that may be known or unknown, such as haemozoin pigment and other toxic factors accumulate in the infected erythrocyte. These are released into the bloodstream when the infected cells lyse and release merozoites that invade other red blood cells (Olivier *et al*., 2014). Furthermore, *P. falciparum-infected* erythrocytes, particularly those with mature trophozoites, adhere to the vascular endothelium of venular blood vessel walls and do not freely circulate in the blood (Rowe *et al*., 2009). When this sequestration of infected erythrocytes occurs in the vessels of the brain, it leads to cerebral malaria, which is associated with high mortality, particularly in children (Idro *et al.*, 2010). In terms of diagnosis and treatment, the evaluation of haematological parameters in malaria patients enable physicians to establish early and efficient therapeutic interventions to avoid major complications (D'acremont *et al*., 2002). According to Jiero *et al*. (2021), patients suffering from malaria infection tend to possess significantly lower lymphocyte, leukocyte, platelet, red blood cell, haemoglobin and eosinophil counts. Knowledge of changes in haematological profile in children infected by malaria can increase malaria diagnosis and thus assist in its treatment in other to prevent death. On the other hand, studies conducted earlier demonstrated that malaria infection is significantly associated with poor nutrition. It has been revealed that inadequate nutrition predisposes children to various infections or make it extremely challenging to recover from infections (Oldenburg *et al*., 2018). Generally, Nigeria is a highly endemic malaria region, and the association between

malaria parasite density, haematological parameters and nutrition is complex and not well understood. Hence, this study was carried out to determine the association between malaria infection and parasite density, haematological profile and nutritional indices among breastfeeding children 0-2 years in Awka, Anambra State, South-eastern Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Amaku, Awka, Anambra State in South Eastern Nigeria. COOUTH is a public hospital, located in Awka, Awka South Local Government Area, Anambra State. It was established on the 8th of August, 2019, and operates on a 24-hour basis. COOUTH Amaku is licensed by the Nigerian Ministry of Health, with facility code 04/06/1/1/1/0039 and registered as a Tertiary Health care centre. Awka is situated in the tropical rainforest zone in south-eastern Nigeria and lies on latitude 6 12'25° N and longitude 7 04'04° E. The temperature of the city is 27°C-30°C from June to December and 32°C-34°C from January to April, with the last few months of dry season marked by intense heat (Ishar *et al*., 2024).

Study Design

This was a hospital-based cross-sectional study and was conducted for a period of one month between July and August 2024.

Study Population

The study population consisted of all the children aged 0-2 years presenting with febrile illness in the outpatient paediatric unit of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital at the time of the survey.

Ethical Consideration

Ethical clearance for this study was sought and obtained from the Health Research Ethics committee of the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State, Nigeria. The ethical approval was assigned reference number (COOUTH/CMAC/ETH.C/HREC//vol.1/136).

Sample size Determination

The sample size for this study was calculated using the sample size for categorical data formula according to Emmanuel *et al*. (2024).

$$n = \frac{P(1-p)Z^2}{d^2}$$

Where **n** is the required sample size, **P** is the percentage occurrence of a state or condition = 5% (Bartlett *et al.*, 2001), **E** is the percentage maximum error required = 4%, **Z** is the value corresponding to level of confidence required = 1.96 (95% confidence level)

$N = 0.05(1-0.05)1.96^2/0.004^2$

$N = 114.0475 \approx 114$

The minimum sample size required for this study has been determined as 114.

Informed Consent

Oral consent was sought and obtained from the parents or guidance of the children upon arrival in the paediatric ward. The aim and objectives of the study were explained to parents and only children whose parents consented were included in the study. Other vital information obtained from the patients were handled with great confidentiality and used only for the purpose of this study.

Inclusion/Exclusion Criteria

Children between the age of 0-2 years who visited the hospital during the study period and whose parents gave their consent were included in the study while children who were above 2 years and whose parents refuse to consent were excluded from the study.

Determination of Malaria Parasites

The diagnosis for malaria infection was done using microscopy. One millilitre of venous blood was collected from each participant. Thick and thin blood smears were made on glass slides and stained using 10% Giemsa stain. The blood films were examined under the light microscope using immersion oil and x100 objective lens. A patient was said to be positive if microscopy demonstrated the presence of malaria parasites.

Use of Questionnaire for Demographic data

A structured pretested questionnaire was administered to collect demographic information (age, gender), anthropometric measurements (height and weight) and breastfeeding pattern of the children. The questionnaire was shared out to the parents or guardians of the children and monitored to ensure accurate completion.

Determination of Haematological Parameters

Venous blood sample was collected from each child using a 2ml syringe and was dispensed into an Ethylene Di-amine Tetra-acetic (EDTA) tube. The EDTA tube was gently shaken to mix the blood with the anticoagulant contained in the tubes to avoid clotting. The haematological parameters of the blood samples were determined by using Automated Hematology Analyzer (Abbott Cell-Dyn 1700 Automated Analyzer) according to manufacturer's guide.

Determination of Anthropometric Measurements

Anthropometric measurement such as height (cm) was determined using a long-calibrated meter rule. On the other hand, children's weight (kg) was measured with the aid of a weighing balance (Salter Model 180, England) for children 0-6 months and Detecto-Medic (Detecto scales inc. Brooklyn, N.Y. U.S.A) for those from >7 months.

Data analysis

Descriptive statistics, such as means, medians, and standard deviations, was used to summarize the main outcome variables (malaria parasite density, haematology, and nutritional indicators) and to assess the distribution and central tendency of data. Inferential statistical tests, such as regression analysis and hypothesis testing, was used to examine the relationships between the variables of interest and to test hypotheses about the associations between malaria parasite density, haematology, and nutritional indicators in breastfeeding children. Regression analysis was used to model the relationships between the variables and to estimate the strength and direction of the associations.

RESULTS AND DISCUSSION

Results

Demographic characteristics of the study population

One hundred and fourteen (N=114) breastfeeding children aged 0-2 years were included in the study at the Chukwuemeka Odumegwu Ojukwu University Teaching Hospital. The sample consisted of 57 males and females, with 31.6%, 39.5%, and 28.9% of the children falling into the 0-6 months, 7-12 months, and 19-24 months age groups, respectively. Notably, no child aged 13-18 months were included in this study. The mean age of the sampled breastfeeding children between 0-2 years was 11.36±9.07 months (Table 1).

Table 1: Demographic characteristics of breastfeeding children at COOUTH

Variable	Number sampled	Per cent (%)
Sex		
Male	57	50.0
Female	57	50.0
Age group (months)		
0-6	36	31.6
7-12	45	39.5
13-18	-	-
19-24	33	28.9
Total	114	100
Mean age ±SD (months)	11.36±9.07	

Breastfeeding pattern of the sample population

Of the 114 breastfeeding children sampled, only 32.5% (n=37) were exclusively given breast milk. Those who received breast milk and water were 15.8% (n=18) while those who received breast milk and other food were 51.8% (n=59), as shown in Table 2.

Table 2: Breastfeeding pattern of the study population at COOUTH

Breastfeeding pattern	Number sampled	Per cent (%)
Exclusive breastfeeding		
Yes	37	32.5
No	77	67.5
Breastfeeding + water		
Yes	18	15.8
No	98	84.2
Breastfeeding + other food		
Yes	59	51.8
No	55	48.2
Total	114	100

Nutritional status of breastfeeding children based on anthropometric characteristics

The height of male breastfed children was significantly higher ($p < 0.05$) than that of their female counterparts (Figure 1). Concerning their weight, there was no significant difference (Figure 2). The height and weight of the children varied significantly ($p<0.05$) based on age group with both height and weight increasing as age increased (Figures 3 and 4).

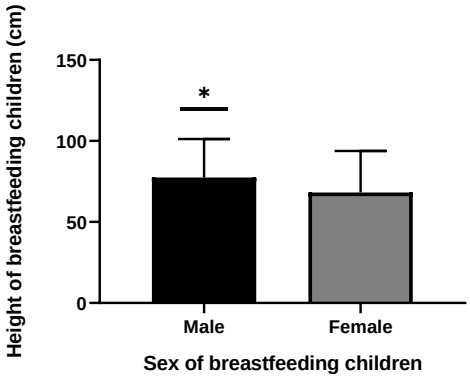

Figure 1: Heights of male and female breastfeeding children

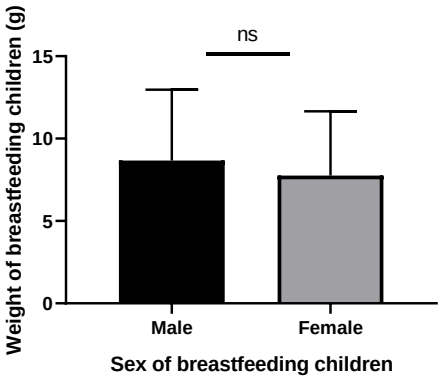

Figure 2: Weight of male and female breastfeeding children

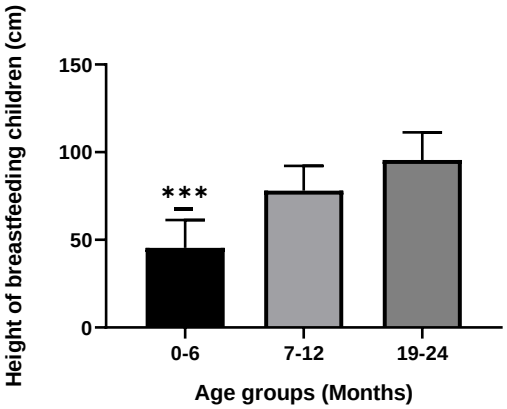

Figure 3: Heights of breastfeeding children of different age groups

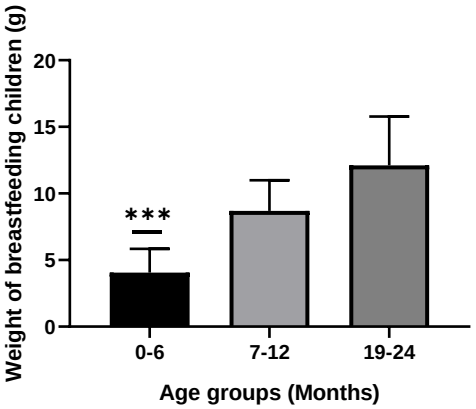

Figure 4: Weights of breastfeeding children of different age groups

Prevalence and parasite density of malaria parasite in the study population

Of the 114 breastfeeding children sampled at COOUTH, 26.3% (n=30) were positive for malaria. The other 73.7% (n=84) did not test positive during screening. There was no significant difference (p>0.05) in the prevalence of malaria parasite infection when the sex and age groups of breastfeeding children were compared (Table 3). Male children had a higher mean parasite density than their female counterparts but the difference was insignificant (p>0.05). Parasite density across age groups increased as age increased, however, the variation in parasite density was not significantly different (p>0.05), as shown in Table 4.

Table 3: Prevalence of malaria parasites with respect to gender and age groups

Variable	Number sampled	Number positive (%)	χ2	P value
Sex				
Male	57	15 (26.3)	0.00	1.000^{ns}
Female	57	15 (26.3)		
Age groups (Months)				
0-6	36	8 (22.7)	0.575	0.747^{ns}
7-12	45	12 (26.7)		
19-24	33	10 (30.3)		

ns: not significant

Table 4: Parasite density of breastfeeding children

Variable	Parasite density	P value
Sex		
Male	4.91±1.82	0.22^{ns}
Female	2.49±0.72	
Age groups (Months)		
0-6	1.58±0.55	0.27^{ns}
7-12	3.96±1.54	
19-24	5.67±2.57	

ns: not significant

Relationship between parasite density and nutritional status of breastfeeding children

There was no significant difference in the height and weight of breastfeeding children based on their malaria infection status. Similarly, there was no correlation between parasite density and the nutritional status of breastfeeding children (Table 5).

Table 5: Association between the malaria parasite and nutritional status of children

Nutritional status of children	Prevalence of malaria parasite			Correlation with parasite density (r)
	Negative	Positive	P-value	
Height (cm)	72.51±25.91	73.70±22.86	0.824^{ns}	0.110^{ns}
Weight (g)	8.07±4.00	8.63±4.44	0.522^{ns}	0.094^{ns}

ns: not significant

Relationship between the haematology and the parasite density, haematology and nutritional indicators of breastfeeding children

A significant negative correlation was found between the height of breastfeeding children and MCV (r= -0.39, p<0.01) as well as MCH (r= -0.22, p<0.05). Additionally, the weight of the children displayed a significant negative association with PCV (r=0.19, p<0.05), MCV (r=-0.34, p<0.01), and EOS_PLA (r= -0.19, p<0.05). The only observed relationship between parasite density and haematological parameters was with WBC (r=0.301, p<0.01), as shown in Table 6.

Table 6: Relationship between haematology, nutritional indicators and parasite density of breastfeeding children

Independent variables	Haematological parameters										
	WBC	**PCV**	**MCV**	**MCH**	**MCHC**	**NEN_LYM**	**LYM_MON**	**MON_GRA**	**EOS_PLA**	**BAS**	**PLAT**
Height (cm)	0.08	-0.18	-0.39**	-0.22*	-0.08	-0.06	0.09	-0.12	-0.17	0.10	0.04
Weight (g)	0.04	-0.19*	-0.34**	-0.16	-0.01	-0.07	0.13	-0.12	-0.19*	-0.11	0.02
Parasite density	0.30**	-0.12	-0.12	0.02	0.10	0.00	-0.13	0.11	0.17	-0.06	0.02

**significant correlation at p<0.01; *significant correlation at p<0.05

Relationship between the pattern of breastfeeding, malaria parasite density and haematology of breastfeeding children

The exclusive breastfeeding of children showed positive correlations with PCV (r=0.21, p<0.05), MCV (r=0.44, p<0.01), and EOS_PLAT (r=0.22, p<0.05). There was no correlation between breastfeeding with water and haematology. However, breastfeeding in combination with other food showed a negative correlation with MCV (r= -0.38, p<0.01) and EOS_PLAT (r= -0.21, p<0.05). The breastfeeding patterns demonstrated no correlation with parasite density (Table 7).

Table 7: Relationship between breastfeeding pattern, haematology and parasite density

Independent variables	Haematological parameters											
	WBC	**PCV**	**MCV**	**MCH**	**MCHC**	**NEN_LYM**	**LYM_MON**	**MON_GRA**	**EOS_PLA**	**BAS**	**PLAT**	**Parasite density**
Exclusive	-0.01	0.21*	0.44**	0.23*	0.11	-0.01	-0.14	0.09	0.22*	0.26	0.05	-0.13
BF+ water	0.09	-0.05	-0.05	-0.08	-0.08	0.03	-0.01	0.03	0.01	-0.05	-0.07	-0.01
BF with other food	-0.06	-0.16	-0.38**	-0.16	-0.05	-0.01	0.13	-0.11	-0.21*	-0.20	0.01	0.13

BF: breastfeeding; **significant correlation at p<0.01; *significant correlation at p<0.05

Discussion

In Nigeria, malaria is hyper-endemic with stable transmission and a mortality rate of about 10% in children aged less than 2 years (Chukwuocha, *et al*., 2009). Malaria parasitaemia in children below 2 years varies from one area to another. The relationship between malaria parasite density, breastfeeding pattern and associated haematological profile of these children is not well understood. In the present study, an overall prevalence of 26.3% of malaria infection was recorded out of the 114 breastfeeding children sampled at COOUTH. This result is similar to the finding of Elechi *et al*. (2015) that reported prevalence of 27% among under 5 children in Maiduguri, northern Nigeria. However, this result is lower than the 47.2% malaria prevalence reported by Okeke *et al*. (2024) in children 0-5 years in Awka, Anambra State and the 52.2% by Oluwafemi *et al*. (2024) in Ondo State, southwestern Nigeria. On the other hand, this result is higher than the 15% documented by Adebisi *et al*. (2018) in Oyo State and 16.9% reported in Lagos State by Oladosu and Oyibo (2013). The severity of malaria disease can vary depending on several factors, including the *Plasmodium* species, the patient's immunity, and the level of parasite density. Although prevalence of malaria infection was similar for all gender (26.3%) each, the higher malaria parasite density observed in male children (4.91±1.82) compared to female children (2.49±0.72) might be indicative of parasite gender preference or different exposure levels of the children to mosquito bites. Parasite density across age groups increased as age increased, however, the variation in parasite density was not significantly different. With the passage of time, young infants have been understood to be relatively protected from malaria and its complications. According to Afolabi *et al*. (2021), young infants possess astonishing level of foetal haemoglobin which makes their red blood cells to be relatively resistant to being infected by the *Plasmodium* parasite. Similarly, the reduced amount of para-amino-benzoic acid (PABA) in the red blood cells of younger children is also protective from malaria as the parasite requires PABA as substrate for rapid proliferation (Ibadin *et al*., 2012). Another factor could be antibodies and lactoferrin from breastmilk coupled with derived maternal malarial antibodies transferred to the baby via the placenta that help to protect these children from *Plasmodium* infection. The above stated facts thus explain the reason for the lower prevalence of malaria infection in younger children compared to slightly grown ones. The exclusive breastfeeding of children showed positive correlations with certain haematological parameters such as PCV, MCV, and EOS_PLAT. On the other hand, there was no association between breastfeeding with water and haematology. However, breastfeeding in combination with other food showed a negative correlation with MCV. A study conducted previously in Kinshasa, Congo by Brazeau *et al*. (2016) showed that exclusive breastfeeding was associated with reduced clinical malaria in infants as well as protection against pyrexia in infants. According to Ballard and Morrow (2013), this reduction in malaria infection susceptibility of exclusively breastfed children might be due to the immunomodulatory effects of breast milk. Breastfeeding is the best source of nutrition for infants and young children, providing the perfect balance of nutrients, antibodies, and enzymes that are essential for healthy growth and development. Breastfeeding has numerous health benefits for both the baby and the mother, and is recommended by the World Health Organization for the first six months of life,

with continued breastfeeding for up to two years or beyond. This finding further contributes to the growing body of research on the impact of breastfeeding in malaria reduction in children and highlights the need for continued efforts to address this important public health challenge. Although, breastfeeding is highly beneficial, it poses challenges and barriers, particularly for low-income and marginalized communities, and it is important to provide support and resources to help women breastfeed successfully.

Nutritional indicators are important measures that can be used to assess the nutritional status of breastfeeding children. The findings of this study showed no association between nutritional indicators such as weight and height with respect to malaria infection. This result is in line with the work of Mmbando *et al.* (2022) who also reported no significant association between nutritional indices and malaria infection. Other studies have reported conflicting results for nutritional indicators, such as weight-for-age, length-for-age, and weight-for-length, in relation to malaria parasite density and haematology. Growth and development are important indicators of nutritional status, while other indicators such as weight-for-age, length-for-age, and weight-for-length, dietary intake, nutrient status, and biochemical markers can also be used to assess the child's nutritional status. These indicators can help healthcare providers to identify children who may be at risk of malnutrition or other health problems and to provide appropriate interventions to address these issues. It is important to monitor these indicators in breastfeeding children who are at risk of malaria to ensure that they receive appropriate care and treatment.

The relationship between malaria parasite density, haematology, and nutritional indicators in breastfeeding children is complex and interrelated. High levels of parasite density can lead to severe malaria, which can cause anaemia and impact the child's nutritional status. Haematology is also influenced by malaria parasite density since changes in the blood profile can have a significant impact on the child's overall health and well-being. Further research is needed to fully understand these relationships to develop effective interventions to improve the health and well-being of breastfeeding children in areas where malaria is endemic.

CONCLUSION

Based on the provided information, it can be concluded that malaria is a significant concern for breastfeeding children in Nigeria, with a considerable proportion testing positive for malaria parasite. The study also revealed that factors other than sex and age may play a more crucial role in determining the risk and severity of malaria infection in breastfeeding children. Additionally, the findings suggest that exclusive breastfeeding practices are not widely observed among the sampled population, indicating a need for improved education and support for exclusive breastfeeding. Furthermore, the study did not find a significant impact of malaria infection on the growth and development of breastfeeding children. These conclusions highlight the importance of addressing malaria prevention and promoting exclusive breastfeeding practices to ensure the well-being of breastfeeding children in Nigeria. It is recommended that regular screening and monitoring should be conducted nationwide. Implementing preventive measures such as insecticide-treated bed nets and antimalarial prophylaxis can help reduce the risk of malaria transmission in children as well as adults. Additionally, ensuring proper nutrition and prompt treatment of suspected malaria cases are crucial for overall health outcomes in this age group.

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