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Molecular Detection of the Effect of Malaria Infection on Nutritional Status in Paediatrics Attending Primary Health Care Hospitals (PHC) Around Sokoto Metropolitan, Sokoto, Nigeria

Jafaru Suleiman, Sani Y. Lema, and Nike T. Isyaku

ABSTRACT

Molecular diagnosis is now considered to be the most efficient and reliable method for detection of malaria parasites; hence the present research was designed to access the effect of malaria infection on the nutritional status of the pediatrics attending PHC in Sokoto, 400 children were recruited randomly, nutritional status was determined using mid-upper armed circumference (MUAC) tape, blood samples was collected then subjected to Nested Polymerase Chain Reaction (nPCR) test. Out of 400 samples analyzed, 199/400 (49.75%) were acutely malnourished, 117/400 (44.25%) were normal and 84/400 (21.00%) were severely malnourished. Although 237 (59.25%,) were found positive for *Plasmodium falciparum*, those that are severely malnourished had the highest infection rate [78/84 (92.86%)] followed by those that are normal [124/199 (62.31%)] than those that were acutely malnourished [35/84 (41.67%)], a significant association was reported for those that are severely malnourished based on parent's occupation (P=0.001), place of living (P=0.000), and sleeping under nets (P=0.012), there was also significant relationship in those that are normally based on gender (P=0.001), and sleeping under nets (P=0.021) only while those that were normal showed significant relationship based on sleeping under nets (P=0.001) and parents occupation (P=0.000). The high prevalence of malaria in the study demonstrates high sensitivity and specificity of molecular diagnosis. There is a need to embark on massive community campaigns and adoption of mosquito control measures in the study area.

Keywords: hospital, molecular, nutrition, pediatrics, plasmodium.

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I. Introduction

Malaria, is disease commonly transmitted when any female Anopheles mosquito carrying *Plasmodium* bite and inoculate the parasites into the susceptible host, is a potentially fatal disease that is spread through; transfusion of infected blood and used of infected needles also, congenital malaria occurs when an infected pregnant woman gives birth to a child who has the disease [1]. When the parasites are released into a person's bloodstream, they travel to the liver where they mature after a few days, infect red blood cells after a few hours, and then multiply to cause the infected cells to burst open, resulting in symptoms that happen in cycles that last for days [2].

Plasmodium falciparum, *P. vivax*, *P. ovale*, and *P. malariae* are the four types of malaria parasites that can affect humans in Africa; however, *P. falciparum* causes serious malaria illness and has a higher risk of mortality [3]

Child malnutrition is linked to about 60% of under-five mortality in Africa, which prevents children from reaching their full physical, mental, and potential health. Physical effects of prolonged states of malnourishment among children include delayed physical growth and motor development, lower intellectual quotient, greater behavioral problems, and deficient social skills [4]. Most studies on child nutritional status have described prevalence of malnutrition among children under five years old and examined socioeconomic, demographic, and cultural factors associated with child malnutrition in Sokoto State. Childhood malnutrition is spatially structured, and rates in underdeveloped countries that rely on the government remain very high [5].

Because of its geography, 97% of Nigeria is at danger for contracting malaria, and the remaining 3% reside in a malaria-free area. In Nigeria, 300,000 people every year die from malaria [6].

Malaria infection among children has had a negative impact on the community in one way or another because it reduces parental absences from work, family caregiving time, lost productivity, cost of treatment, which includes transportation, and premature mortality when children are diagnosed with the disease [7].

RDT and Microscopy are the most widely used Techniques for detection of malaria parasites in Sokoto though with certain limitations; In the case of RDT, the dye-labelled antibody binds to a parasite antigen and then the resulting complex is caught on the strip by a band of bound antibody that produces a visible line, which has various limitations in terms of accurate diagnosis of malaria [8]. Similar to microscopy, particularly in terms of variable levels of sensitivity for various goods [9].

The nutritional status of a person with malaria is the biggest factor in host resistance and recovery and it is the main factor of nutritional issues that are involved with malaria. Malaria may increase the incidence and severity of malnutrition, and malnutrition may increase the risk of infection with the disease [10].

Recognizing whether nutrition and health are related is vital because doing so may help communities become more economically stable and lower the incidence of malaria, both of which are crucial steps toward improving health [11].

Because molecular biological setup in clinical settings is not always feasible, PCR diagnosis is currently restricted to laboratory-based diagnosis and serves as a useful tool for epidemiological understanding of malaria infections. In recent years, the PCR technique in amplifying the Plasmodium species' genes was found to be superior to the traditional microscopy and RDT in detecting malaria parasites [12]–[14].

By giving information on the relationship between malaria infection and children's nutritional status in several communities around the Sokoto metropolis, and molecular level, the current research is intended to help control malaria infections among children.

II. Methodology

A. Study Area

Sokoto is located at the North-western Nigeria with latitude 5.247552 and longitude 13.005873 east. Sokoto State is bordered to the north by the Republic of Niger, to the south by Kebbi State, and to the southeast by Zamfara State. The population of the state, which has a total land area of around 25,973 sq. km, was 3,702,676 in 2006 and is expected to reach 5,138,829 in 2019. It is located in a region of short grass savannah flora, bushes, and a usually dry climate that progressively transitions into the desert over the Niger Republic's border. From mid-June to mid-September, there is little rain, and from November to February, there is a dry, dusty wind.

B. Sample Size Estimation

1) Sample size/population of the study

Sample size was estimated using a formula described by Bluman, [15].

$$N = \frac{Z2\,p\,q}{d2}$$

where:
N – minimum sample size
Z – confidence interval: 1.96
P = Probability of success: 0.5
q = Probability of failure: 1-p = 0.5 and
d = Degree of accuracy desire=0.05.

Therefore

$$N = \frac{1.96X1.96X0.5X0.5}{(0.05)2}$$
$$= \frac{3.842X0.5X0.5}{0.0025}$$
$$= 384 \approx 400$$
$$\frac{400}{4} = 100$$

A total number of 400 children were subjected into the study; Each 100 from Four (4) Primary Health Cares, namely: Yahaya Abdulkarim Primary Health Care, Kofar Kade, Dallatu Primary Health Care, Tudun Wada, Gagi Primary Health Care Gagi, and Ibrahim Gusau Primary Health Care, Minanata

C. Ethical Consideration

A letter of introduction was obtained from the head of the biological sciences department at Sokoto State University, and it was combined with a research proposal before being sent to the director of the ethical research committee at the Sokoto State Ministry of Health, which gave its approval for the study.

D. Informed Consent

Prior to the study's start, the participant's parents provided their informed consent. They were informed of the study's purpose and objectives as well as its significance, and they were made aware that their privacy would be always respected. Individuals who refused to participate were not included in the survey.

E. Detection of Nutritional Status

In this study MUAC tape was used in determining the Nutritional Status of the children. The tape was placed at the mid of the left upper arm to measure the circumference; when the circumference line showed the red color, the subject was considered as an indication of severe malnutrition (SAM); yellow colour, indicates that the child is at risk for acute malnutrition (AM); while green colour, indicates that the child is well nourished (N) (Fig. 1).

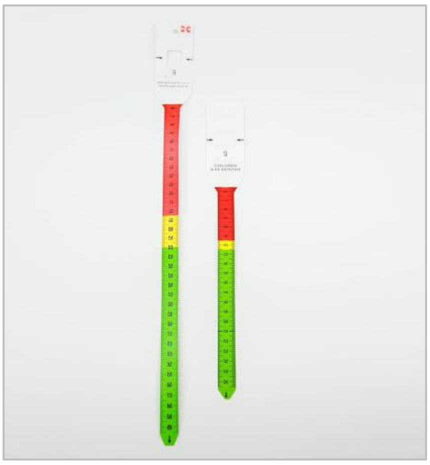

Fig. 1. Mid upper armed circumference tape.

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F. Sample Analysis

Blood samples were used to extract genomic DNA in accordance with the manufacturer's instructions. Following the nPCR amplification techniques outlined by Snounou *et al*. [16] the molecular identification of the *Plasmodium* species was carried out. A negative control made up of sterile, double-distilled water served as the positive control in each experiment. Thermal Fisher Scientific, Waltham, Massachusetts, USA, provided the Dream Taq DNA polymerase kit, and PCR was carried out using it in accordance with the manufacturer's instructions. The PCR results were examined using electrophoresis on 1% agarose gel with ethidium bromide and ultraviolet (UV) light visualization [17]

G. Data Analysis

Data were subjected to descriptive statistical analysis using percentages in determining the prevalence rates; Binary logistic regression analysis was used to identify the association of the effect of malaria infection on the nutritional status of the children in the study area at $P<0.05$.

III. Results

A. Nutritional Status of the Pediatrics in the Study Area

Out of 400 samples analyzed, for nutritional status, it was observed that 49.8% were acutely malnourished, 29.2% were normal and 21.0% were severely malnourished as shown in Fig. 2.

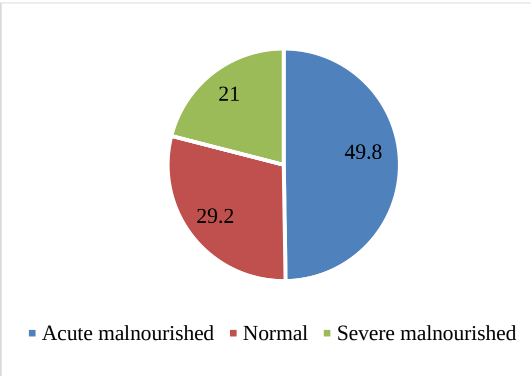

Fig. 2. Nutritional status of pediatrics in the study areas.

B. Prevalence of Malaria Among the Pediatrics Based on their Nutritional Status

Out of four hundred samples analyzed, 237 were found infected with Plasmodium falciparum, given the prevalence of 59.25%; severely malnourished children recorded the highest infection rate of 92.9% followed by those that were normal (62.3%), then those that were acutely malnourished (29.9%). (Table I) There was a significant association between the infection-based nutritional status.

C. Effect of Malaria Among the Children with Normal Nutritional Status in the Study Area

Results of malaria infection among pediatrics with normal nutritional status showed that; males were more highly infected (64.1%) than females (60.8%) based on gender. However, there was no significant association ($p=0.216$).

TABLE I: Distribution of Malaria Infection among the Participants in the Study Area Based on Their Nutritional Status

Nutritional Status	Number Examined	Number Positives	Prevalence (%)
Severely Malnourished	84	78	92.9
Normal	199	124	62.3
Acute Malnourished	117	35	29.9
Total	400	237	59.3

Regarding the aged group children aged<24 months were highly infected (77.1%) followed by those aged between 49–72 months (58.1%), against those aged ranges between 25–48 months with an infection rate of 57.3%, and least prevalence of 45.0% was recorded among the aged group with>73 months, there were no significant association based on age groups ($p=0.482$).

Highly significantly associated infection rate ($p=0.000$) was observed among the children of farmers (80.9%), followed by children of businessmen with a 68.9% infection rate, then children of civil servants (50.0%), while least infection rate of 44.4% was observed among the children of parents with other occupations.

Based on the place of living, it was reviled that, those children from Dange Shuni Areas recorded the highest infection rate of 93.5%, followed by those from Wamakko (73.1%), then those living around areas of Sokoto South (56.9%), while least infection rate was recorded among the children living around Sokoto North areas of Sokoto metropolis (49.2%). There was no significant association based on the Place of living around the Sokoto metropolis ($P=0.421$).

Children that were not sleeping under the net were highly infected (79.3%) than those that were sleeping under the net (49.1%) with a statistically significant association ($p=0.001$) as shown in Table II.

TABLE II: Prevalence of Malaria among the Children with Normal Nutritional Status Based on Risk the Factors

Risk Factors	No. Examined	No. Positive	Prevalence (%)	P-Value
Gender				
Male	92	59	64.1	0.216
Female	107	65	60.8	
Age (Months)				
≤24	61	47	77.1	0.482
49–72	43	25	58.1	
25–48	75	43	57.3	
≥73	20	09	45.0	
Parents occupation				
Farmers	63	51	80.9	0.000
Businessmen	29	20	68.9	
Civil Servants	98	49	50.0	
Others	09	4	44.4	
Place of living				
Dange Shuni	31	29	93.5	0.421
Wamakko	26	19	73.1	
Sokoto South	79	45	56.9	
Sokoto North	63	31	49.2	
Sleeping Under the Net				
No	87	69	79.3	0.001
Yes	112	55	49.1	
Total	199	124	62.3	

D. Effect of Malaria Infection Among the Children with Acute Malnutrition in the Study Area

The effect of malaria infection on children with acute malnutrition in the study area showed that; males were more highly infected (35.2%) than females (25.4%). Statistically, a significant association was observed (P=0.001) based on gender.

The infection rate decreases with an increase in the ages of the subjects because children with<24 months had the highest infection rate of 33.3%, followed by those aged between 25–48 months (30.43%) then those aged between 49–72 months (30.2%), while those with aged>73 months had the least infection rate of 28.21%. There was no significant association (p=0.921) based on the age groups of the children in the study area.

Presently, it was reported that children of Businessmen were the most infected group with a prevalence rate of 31.0%, followed by children of farmers (29.7%), then children of civil servants (29.6%), however children of parents with other business had the lowest infection rate of 29.2%. There was no significant association between infection rates of malaria among the children with acute malnutrition in the study area (P=0.241).

Research findings from the study area showed; that children from Sokoto South had the highest infection rate of 32.3%, followed by those from Sokoto North (30.8%), then those from Dange Shuni Areas (28.6%), however, children from Dange Shuni Areas account for the lowest infection rate of 28.1% and there was no significant association for the malaria infection based on the residence areas (P=0.592).

Those children with acute malnutrition and also not sleeping under the mosquito net recorded the highest infection rates of 36.1% than those that were acutely malnourished but sleeping under the net (12.9%). A statistically significant association was observed based on sleeping under the net (P=0.021) (Table III).

E. Status of Malaria Infection Among the Children with Severe Malnutrition in the Study Area

Considering the malaria infection among the children with severe malnutrition in the study area; it was observed that, females had a higher infection rate (93.9%) than males (92.2%) with no significant association (p=0.214)

Similarly, the infection rate was observed to be highest among the children aged between 25–48 months (95.8%) followed by those with ages ranging between 49–72 months (9.0%) than those aged<24 months (93.1%) while children with aged>73 months recorded least infection rate of 90.0%. There was no significant association based on age (P=0.148).

With regards to the parents' occupations of the subjects, the highest prevalence of 96.8% was observed among the children of farmers, then children of businessmen with an infection rate of 95.0%, followed by children of parents with other occupations (90.9%) while children of civil servants had the lowest prevalence of 86.4%. There was a significant association between the prevalence of malaria among children with severe malnutrition based on their parents' occupation (P=0.001).

TABLE III: Effect of Malaria Infection among the Children with Acute Malnutrition in the Study Area

Risk Factors	No. Examined	No. Positive	Prevalence (%)	P-Value
Gender				
Male	54	19	35.2	0.001
Female	63	16	25.4	
Age (Months)				
≤24	12	04	33.3	0.921
25–48	23	07	30.4	
49–72	43	13	30.2	
≥73	39	11	28.2	
Parents occupations				
Businessmen	29	09	31.0	0.241
Farmers	37	11	29.7	
Civil Servants	27	08	29.6	
Others	24	07	29.2	
Place of living				
Sokoto South	31	10	32.3	0.592
Sokoto North	26	08	30.8	
Dange Shuni	28	08	28.6	
Wamakko	32	09	28.1	
Sleeping Under the Net				
No	86	31	36.1	0.021
Yes	31	04	12.9	
Total	117	35	29.9	

The status of malaria among the children in the study area with regards to the place of living, clearly reviled that, children that were from areas of Wamakko had the highest infection rate (96.6%), followed by those that are from Sokoto South areas (93.3%), then those residing around the areas of Dange Shuni (92.9%), then those that were living in Sokoto North Areas (83.3%), Significant association was observed based on residing areas of the subject (P=0.000).

TABLE IV: Effect of Malaria Infection among the Children with Severe Malnutrition with Regards to Risk Factors

Risk Factors	No. Examined	No. Positive	Prevalence (%)	P-Value
Gender				
Female	33	31	93.9	0.214
Male	51	47	92.2	
Age (Months)				
25 – 48	24	23	95.8	0.148
49 – 72	21	19	95.0	
≤ 24	29	27	93.1	
≥ 73	10	09	90.0	
Parents occupations				
Farmers	31	30	96.8	0.001
Businessmen	20	19	95.0	
Others	11	10	90.9	
Civil Servants	22	19	86.4	
Place of living				
Wamakko	29	28	96.6	0.000
Sokoto South	15	14	93.3	
Dange Shuni	28	26	92.9	
Sokoto North	12	10	83.3	
Sleeping Under the Net				
No	69	67	97.1	0.012
Yes	15	11	73.3	
Total	84	78	92.9	

Based on sleeping under the net, it was observed that children who were not sleeping under a mosquito net had a significantly (P=0.012) highest infection rate of 97.1%, while those that were sleeping under the net had a prevalence of 73.3% as shown in Table IV.

IV. Discussion

Children are particularly sensitive to malaria and bear the disease's highest morbidity and mortality loads, making it one of the major public health issues in many developing nations. [19]. Malaria frequently occurs alongside other illnesses and low socioeconomic position, and these factors have further hampered the growth of the affected communities. One of the most prevalent and concerning illnesses, malnutrition hinders children's growth and increases the severity of other health disorders [20].

According to the current research, 59.25% of the study's participants were discovered to have malaria parasites in their blood, which was a higher infection rate than the 58.2% prevalence recorded [21].

In this study, it was found that children's nutritional condition and malaria infection were related, with children with severe malnutrition showing noticeably greater infection rates than those with acute malnutrition. In contrast to the results of Catherine *et al*. [18], who discovered that the incidence of malaria was unrelated to dietary status. However, baseline-treated malaria infection led to greater weight gain and slower linear growth.

The current survey found a link between severe and acute malnutrition and a higher risk of childhood malaria infection, suggesting that poor nutritional status may predispose children to malaria infection because malnutrition-related immune response impairments exist [22]–[24,]. However, the connection between nutritional status and the incidence of malaria has yielded conflicting results in prospective research [25]–[27]. This contradicts the findings of Nwaorgu and Orajaka [18]. who found that although malaria infection is more common in children with chronic malnutrition, the infection incidence increases linearly with decreasing age. They conducted their research in the Awka North Local Government of Anambra State.

Children of farmers with normal nutritional status were found to have higher rates of significantly associated infection; this may be indicative of their parent's occupations, which include dumping grasses and raising animals, which may increase the number of mosquito breeding sites and increase the susceptibility of the children to infection with plasmodia when the mosquitoes sucking their blood. This was consistent with the findings of Alexandre *et al*. [28], who claimed that farming was one of the risk factors that predisposed people and households to contract malaria parasites due to poor environmental sanitation, farming practices such as excretory product disposal, and personal hygiene issues among farmers.

The findings of Okiring *et al*. [29] who claimed that the female gender was linked to a higher incidence of malaria across all individuals contradict the findings of this study, which found that males with acute malnutrition are substantially more likely to be infected than females. This was also in disagreement with Shankar *et al*. [30], findings, which claimed that sex had no bearing on the prevalence among children. Children who are refugees and internally displaced people are particularly susceptible to malaria. In the 1990s, malaria in refugee camps was a significant issue.

In children with malnutrition and normal nutritional status, not sleeping under an insecticide-treated net (ITN) is a risk factor and an indicator of higher infection that may be related to an underlying immune response impairment [31], which has been linked to increased risks of malaria [32], [33]. Because many of the study's children did not use a mosquito net while they slept, the number of infected children in the research region was higher in the current survey's analysis of the relationship between not using a net while sleeping and malaria incidence.

Finally, cross-sectional studies have shown a link between poor nutritional indicators and a higher risk of malaria, indicating that malnutrition-related immune response impairment may predispose children to malaria infection [34].

V. Conclusion

The present study showed that majority of the respondent were acutely malnourished and higher sensitivity and specificity of nPCR as a suggestive indicates the highest effect of malaria infection among the children with severe malnutrition, followed by those with acute malnutrition while those with normal nutritional status present least infection. The effect of malaria infection was observed to be significant for those that are sleeping under net, parents' occupations and place of living

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