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Assessment of Immunoglobulin Response to *Plasmodium falciparum* Infection among Children attending Murtala Muhammad Specialist Hospital Kano, Kano State

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Abstract
Globally, malaria fever is a public major health problem which is mostly prevalent in 97 countries and territories in the subtropics and tropics. The aim of this study, was to determine the immunoglobulin (IgG and IgM) responses and assess them in both male and female children between the age group of 1-10 years that were infested with Plasmodium falciparum. Malaria [RDT] kit was used for qualitative immunochromatographic flow tests in a dipstick (strip) or cassette forms that detect malaria antigen present in peripheral blood. The ELISA test kit was used for the qualitative determination of antibodies against Plasmodium in human serum for accurate qualitative measurement of the antibodies produced. The specimens were analysed in the Laboratory of the Department of Medical Laboratory Science, Murtala Muhammad Specialist Hospital Kano State. A total of 350 children with Plasmodium falciparum, male 190(54.3%) and female 160(45.7%) with 100 control involving of both males and females not greater than 10 years old were recorded. The comparative mean value and standard deviation Immunoglobulin G and Immunoglobulin M of the patients and control were significantly high (P<0.05). The relationship between the age distribution and Immunoglobulin of male patients for Immunoglobulin G and Immunoglobulin M were insignificant (P>0.05) while that of female patients were not significant (P>0.05). However, in this study the immunological response of IgM was predominant among children infected with Plasmodium falciparum. Effective prevention and control measures should be employed for the control of malaria in the community.

Keywords: Blood, Children, IgG, IgM, *Plasmodium falciparum*.

INTRODUCTION
Malaria is a protozoan infection with one or more of Plasmodium species e.g., *Plasmodium falciparum* gotten from the bite of female mosquito (Anopheles) in an individual (Rayner *et al.,* 2011). *Plasmodium falciparum* is the severe strain of the malaria species compared with every malarial disease. The other species that causes malaria include *Plasmodium vivax, Plasmodium ovale, and Plasmodium malariae* (Paul *et. al.,* 2004).

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Humans are infected by a female anopheles mosquito, which transfers a vector of parasite (mostly *Plasmodium falciparum)* via its saliva into the blood stream, causing malaria. The parasite normally infest the liver where it undergoes asexual reproduction and then penetrates the red blood cells where multiple circle of replication takes place (Miller *et. al.,* 2002; Mikhail *et. al.,* 2010)

This species of *Plasmodium falciparum* procreate the most delirious form of malaria disease. It has higher medical disease condition and often fatal in the whole community. Malaria infection claims more lives especially of children than the adults compared to any other disease (Rich *et al.,* 2009).

Immunoglobulin G (IgG) antibodies are the smallest antibodies found in all body fluids. They constitute about 75 to 80% of the total antibodies in human (Andrian, 2016). Immunoglobulin G antibodies are very essential against antigens and are the only antibodies that can cross the placenta in a gravid mother and help to protect her fetus (Hashira *et al.,* 2000). Immunoglobulin G, an antibody, has two binding sites of antigen which represent about75% of human serum antibodies. Immunoglobulin G is the common antibody found in the blood, it is created and released by plasma B cells (Hashira *et al.,* 2000).

They can be formed in blood and lymph nodes, and are the first generation of antibody produced against antigens. They stimulate other defense systems of the body to destroy antigenic substances (Recine *et al.,* 2011). It is known as the largest antibody in the human circulatory system (Recine *et al.,* 2011).
The aim of the study is to determine the assessment of immunoglobulin response to *Plasmodium falciparum* infection among Children attending Murtala Muhammad Specialist Hospital Kano, Kano State,

MATERIALS AND METHOD

Study Area

The study was performed at the Murtala Muhammed Specialist Hospital was established about 94 years ago, and is one of the largest medical facilities in Africa. Murtala Muhammad Specialist Hospital that is located at kofar mata road, in Kano Municipal local government area within Kano metropolis. The selected hospital is reference hospital in the state where people from various parts of the state and neighboring states of various occupations attend. The hospital gives more than 70% of health care delivery in the state at large.

Metropolitan Kano encompasses all the eight local governments of Dala, Fagge, Gwale, Municipal, Nassarawa, Tarauni, part of Kumbotso and Ungogo. This is in addition to part of local governments which was integrated in to local metropolis for planning purposes. It lies from Latitudes 11^0 52'N to 12^0 7'N and Longitudes 8^0 22.5`E to 8^0 47'E and is 1549ft above sea level. Kano metropolis is bounded by Minjibir LGA on the North East and Gezawa LGA to the East. While, Dawakin Kudu LGA to the South East, Madobi and Tofa LGA's to the South West and lastly Dawakin Tofa LGA to the North West. The climate of Kano is typical dry and wet climate. Annual rainfall is about 850 – 870mm. The temperature is averagely warm to hot throughout the year at about 27^0C (Olofin *et al.,* 2008).

Ethical Consideration

Ethical approval for this study was obtained from the Operational Research and Advisory Committee, Ministry of Health Kano State.

Collection of Samples

The survey was focused on children between the aged groups of 1-10 years infected with *Plasmodium falciparum*. Controls samples were apparently healthy children without signs and symptoms of malarial infection for both males and females (WHO, 2015). Five milliliters (5mls) of blood sample was collected aseptically and transferred into 0.5ml of aqueous tri-sodium citrate anticoagulant 3.2g/l bottles and mix appropriately. Samples were centrifuged at 1200g for 15minutes and obtained plasma. Plasma was stored at 4^0C until ready for analysis (WHO, 2015).

Methods of Analysis

Enzymes Link Immunosorbent Assay (Elisa) Technique: ELISA test kit of malaria is used for the qualitative determination of antibodies against *Plasmodium* in human serum or plasma (citrate) for accurate qualitative measurement of IgG and IgM antibodies (Holding, 2001).

Procedure

Sample Preparation: All plasma samples were diluted with the sample diluents before analysis, by adding 10µL of the sample to 1000µL of diluents and was then mixed thoroughly with a vortex.

About100µl of controls and samples were added to appropriate micro-plate wells and first well was left as substrate blank. Wells were covered with the foil provided in the kit and incubated at 37°C for 1 hour. Wells were washed three times with 300µL each of washing solution. After the last wash, the plate was decanted and blotted on clean paper towels to remove excess liquid.100µL Malaria HRP conjugate was added into all wells except for the blank. It was incubated for 30 minutes again at room temperature. Step 3 was repeated. 100µL S substrate S solution (TMB) was added into all wells. And it was incubated for 15 minutes in the dark at room temperature. 100µL stop solution was added into all wells in ascending order and at the same rate as for the TMB substrate solution. Specimen was measured at 450nm absorbance within 30 minutes after adding of the stop solution (Cheesbrough, 2006).

Rapid Diagnostic Test (RDT)

Malaria RDTs kits are qualitative immunochromatographic flow tests in a dipstick (strip) or cassette forms that detect malaria antigen present in peripheral blood (Cheesbrough, 2006). Five microliter (5µl) of whole blood was added in to the "S" well. 60µl assay buffer solution (3drops for vial) was added in to the "A" well and timer was started immediately. Result was read in 20 minutes (Cheesbrough, 2006).

RESULTS

A total of 350 children with *P. falciparum,* 190 (54.3%) male and 160 (45.7%) female with 100 control subjects both male and female were recruited in this study aged from 1 – 10 years. Table 1 below, shows the comparative mean value and standard deviation of male Immunoglobulin G and Immunoglobulin M of *P. falciparum* patients and control were significantly high ($P<0.05$).

The comparative mean value and standard deviation of female Immunoglobulin G and M of *P. falciparum* malaria patients and control were also significantly high ($P<0.05$) (Table 2).From Table 3 below the comparative mean value and standard deviation of Immunoglobulin G and M between male and female *P. falciparum* patients and at all level insignificant ($P>0.05$).

Table 1: Comparative mean and standard deviation of Immunoglobulin G and M among male *Plasmodium falciparum* Patient and control subjects.

Test Subjects	N	IgG (NTU) mean±SD	IgM (NTU) mean±SD
Patient	190	18.4±8.87	62.8±23.85
Control	56	3.3±0.98	5.9±1.99
F-ratio		80.831	142.50
P-value		*	*
		0.0001	0.0001

NTU = Nephelometric Turbidity Units; % = Percentage of total number of Specimens analyzed (350); Not significant at <0.05

Table 2: Comparative mean and standard deviation on Immunoglobulin G and M among female *Plasmodium falciparum* Patient and control subjects.

Test Subjects	N	IgG (NTU) mean±SD	IgM (NTU) mean±SD
Patient	160	18.1±8.89	62.6±22.63
Control	44	3.4±1.34	5.6±2.25
F-ratio		44.036	100.75
P-value		*	*
		0.0001	0.0001

NTU = Nephelometric Turbidity Units; % = Percentage of total number of Specimens analyzed (350); Not significant at <0.05

Table 3: Comparative mean and standard deviation of Immunoglobulin G and M between male and female *Plasmodium falciparum* Patients.

Test Subjects	N	IgG (NTU) mean±SD	IgM (NTU) mean±SD
Male	190	18.4±8.87	62.8±23.85
Female	160	18.1±8.89	62.6±22.63
F-ratio		1.006	1.099
P-value		0.7757	0.9386

NTU = Nephelometric Turbidity Units; % = Percentage of total number of Specimens analyzed (350); Not significant at <0.05

Table 4 below shows relationship between the age distribution and Immunoglobulin of male *P. falciparum* patients for Immunoglobulin G and Immunoglobulin M were insignificant (P>0.05).

From Table 5 below the relationship between the age distribution and Immunoglobulin of female *P. falciparum* patients for Immunoglobulin G and M were not significant (P>0.05).

Table 4: Relationship between age distribution and Immunoglobulin among male *Plasmodium falciparum* Patients.

Age distribution (years)	N	IgG (NTU) mean±SD	IgM (NTU) mean±SD
1 - 2	7	17.1±2.55	74.4±21.70
3 – 4	17	17.7±5.12	64.2±27.43
5 – 6	19	17.1±5.12	63.0±23.96
7 – 8	35	20.4±13.0	58.1±23.75
9 -10	17	17.3±5.50	66.2±20.31
F-ratio		0.6733	0.8626
P-value		0.6122	0.4897

Key: *NTU = Nephelometric Turbidity Units; % = Percentage of total number of Specimens analyzed (350); Not significant at <0.05*

Table 5: Relationship between age distribution and Immunoglobulin among female *Plasmodium falciparum* Patients.

Age group (years)	N	IgG (NTU) mean±SD	IgM (NTU) mean±SD
1 - 2	9	17.4±4.95	71.1±19.53
3 – 4	22	18.3±9.42	62.1±21.71
5 – 6	17	19.6±14.24	56.2±26.11
7 – 8	21	16.3±5.97	66.6±23.73
9 -10	11	18.0±3.75	55.8±19.13
F-ratio		0.3246	1.056
P-value		0.8595	0.3845

Key: *NTU = Nephelometric Turbidity Units; % = Percentage of total number of Specimens analyzed (350); Not significant at <0.05*

DISCUSSION

This species of *Plasmodium falciparum* causes the most hazardous form of malaria disease. It has the uppermost complication rates of mortality. *Plasmodium falciparum* is the highest severe strain of the malaria species compared with every malarial disease (Rich *et al.,* 2009).In this study, we observed (60.6%) cases of anemia among children infected with malarial disease. This result shows consistent with earlier findings by (Rayner *et al.,* 2011) who reported 87.5%, and (Agravat and Dhruva, 2010) who found 93% cases of anemic patients during *Plasmodium falciparum* infection in children respectively. Although, the cases of anemia observed shows malaria it's one of the common infection that induced iron deficiency anemia of either hypochromic and/or microcytic type of anemia, absence of iron decreases heme synthesis (*Hardison, 2012*).

The study shows (96%) children infected with malaria responses to IgM were observed with higher percentage than IgG (4%) to *Plasmodium falciparum*. And this indicates most of the patient responses to current/recent infection of malaria disease and was consistent with the previous result obtained by Rowe *et al.* (2002) and McAlister *et al.* (2004). However, it contradicts the findings by Nmorsi *et al.* (2008) among children with malaria diseases in Franceville, Gabon and Ekpoma, Nigeria Countries respectively.

In this study different sets of groups of patients (male and female) infected with *P. falciparum* their means and standard deviation obtained were compared with their control subjects that lead to the following observation: The levels of IgG and IgM responses in both female and male against *P. falciparum* antigens in comparisons with controls subjects were significantly

increased (P<0.01). This is in accordance with similar finding by Andr´e*et al.* (2011). However, in this study, it was observed that there is insignificant responses (P>0.05) in comparisons of mean and standard deviation of male and female IgG and IgM respectively. This can also be attributed to the geographical location of the study population, since the levels of immunoglobulin of malaria infection is seen to vary from one geographic location to another (Babacar *et al.,* 2010).

The immunoglobulin (IgG and IgM) levels among various age groups with malaria infection indicated insignificant responses in both male and female respectively, although IgG was observed to be higher in the age group (7 – 8years) and (5 – 6years) among male and female respectively. This kindly shows that, this particular age groups may be prone to several exposures to malaria infection so frequently that make them to acquire partial immunity (Mallery *et al.,* 2010). Because, the children of these groups they can be stubborn enough not to be kept indoors or by the use of long lasting insecticidal nets (LLINs) when necessary (Murphy, 2016).

CONCLUSION AND RECOMMENDATIONS

The outcome of this study shows the immunological response of IgM was the predominant immunoglobulin obtained among children infected with *Plasmodium falciparum*. It indicated the influence of IgM on malaria disease, despite some category group response to IgG on patients. Our findings shows that malaria infection is associated with the risk of developing anaemia and equally to the immunosuppression of an individual. It is recommended that early diagnosis of malaria including prompt investigation of anemia will provides important information in protecting children from developing hypoglycemia, severe anemia and cerebral malaria that may lead to convulsion and even death (Nmadu, 2015; Murphy, 2016).

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