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Research Paper

CYTOCHEMICAL REACTION OF *Plasmodium sp.* AMONG PATIENTS THAT ARE POSITIVE FOR *Plasmodiasis* AT NIGERIAN AIR FORCE HOSPITAL MAKURDI, BENUE STATE

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Abstract

Aim: *The study was carried out to determine the cytochemical reaction of plasmodium species among patients positive for plasmodiasis, and its prevalence among patients at 161 Nigerian Air Force Hospital Makurdi, Benue State.*

Materials and Methods: *The study was carried out from September, 2019 to November 2019. 200 positive cases where studied using thick and thin blood film stained by Giemsa staining method. Out of the 200 positive cases, 188 were caused by P. falciparum representing 94%, 10 were caused by P. ovale representing 5%, 2 were caused by P. malariae representing 1%. There was no case of P. vivax infection recorded. Subjects were grouped into ranks as officers, soldiers, civilians and lastly sons and daughters of officers and soldiers.*

Results: *Prevalence of infection base on rank revealed that sons and daughters of officers and soldiers recorded the highest number of infected subjects (33%) while still bearing the highest P. falciparum burden (31%). Officers recorded the lowest number of infected subjects (20.5%) though*

soldiers recorded the least P. falciparum burden (19%). These results were made possible following examination of samples using kites, and microscopy after a cytochemical reaction with giemsa dye and the thinned blood smear.

Conclusion: It was established that *P. falciparum* plasmodiasis is the most prevalent infection in the study area with a prevalence of 94%. Infection rate was higher in subjects less than 18 years old (33%).

Key words: *Cytochemical, Giemsa, Patients, Plasmodium, Plasmodiasis*.

INTRODUCTION

Plasmodium, a genus of parasitic protozoans of the sporozoan subclass *Coccidia,* is the causative organisms of plasmodiasis [1]. Plasmodium, which infects red blood cells in mammals (including humans), birds, and reptiles, occur worldwide, especially in tropical and temperate zones. Insect hosts are most frequently mosquitoes of the genera Culex and Anopheles. Vertebrate hosts include reptiles, birds, and mammals [1]. Five species cause human plasmodiasis: *Plasmodium falciparum, Plasmodium malariae, Plasmodium vivax, Plasmodium ovale* and *Plasmodium knowlesi* [2].

Falciparum plasmodiasis is potentially life-threatening. Patients with severe falciparum plasmodiasis may develop liver and kidney failure, convulsions, and coma. Although occasionally severe, infections with *P. vivax* and *P. ovale* generally cause less serious illness, but the parasites can remain dormant in the liver for many months, causing a reappearance of symptoms months or even years later [2]. Plasmodium species exhibit three life-cycle stages— gametocytes, sporozoites, and merozoites. Gametocytes within a mosquito develop into sporozoites. The sporozoites are transmitted via the saliva of a feeding mosquito to the human bloodstream. From there they enter liver parenchyma cells, where they divide and form merozoites [3]. The merozoites are released into the bloodstream and infect red blood cells. Rapid division of the merozoites results in the destruction of the red blood cells, and the newly multiplied merozoites then infect new red blood cells. The red blood cells destroyed by the merozoites liberate toxins that cause the periodic chill-and-fever cycles that are the typical symptoms of *plasmodiasis* [4].

Taxonomy of Plasmodium Parasite

Plasmodium belongs to the phylum *Apicomplexa,* a taxonomic group of single-celled parasites with characteristic secretory organelles at one end of the cell [4]. Within *Apicomplexa*, Plasmodium is within the order *Haemosporida*, a group that includes all apicomplexans that live within blood cells.Based on the presence of the pigment

hemozoin and the method of asexual reproduction, the order is further split into four families, of which Plasmodium is in the family *Plasmodiidae* [5].The genus Plasmodium consists of over 200 species, generally described on the basis of their appearance in blood smears of infected vertebrates [6].

Geographical Distribution of Plasmodium Parasite

Plasmodium falciparum: found in tropical and subtropical areas; major contributor to deaths from severe *plasmodiasis.*

P. vivax: found in Asia and Latin America; has a dormant stage that can cause relapses

P. ovale: found in Africa and the Pacific islands

P. malariae: worldwide; can cause a chronic infection

P. knowlesi: found throughout Southeast Asia; can rapidly progress from an uncomplicated case to a severe *plasmodiasis* infection [7].

Plasmodium falciparum is the most prevalent plasmodium parasite in the WHO African Region, accounting for 99.7% of estimated *plasmodiasis* cases in 2017, as well as in the WHO regions of South-East Asia (62.8%), the Eastern Mediterranean (69%) and the Western Pacific (71.9%). *P. vivax* is the predominant parasite in the WHO Region of the Americas, representing 74.1% of plasmodiasis cases [8].Nigeria contributes 27 per cent of the 216 million plasmodiasis cases and 24 per cent of the 445,000 plasmodiasis deaths. About three out of 10 persons having plasmodiasis in the world live in Nigeria; 1 out of 4 deaths from plasmodiasis globally occur in Nigeria and over 54 million plasmodiasis cases recorded annually for the last three years" [9].Despite some of the successes recorded in the prevention and treatment of *plasmodiasis*, millions of Nigerians and communities are still ravaged by the disease [9] and the main epidemiological factor to Plasmodium falciparum infection in patients should be considered in relation to the endemic Malaria conditions under which people are living according to Okorie *et al.* [19]. Therefore there is need to extend this study to our military base that accommodate more than ten thousand households. Hence different species of plasmodium causes severe or mild forms of plasmodiasis and requires different chemotherapy, this research help determine the major causative specie of plasmodium of causing plasmodiasis in the study area. This will aid in the treatment of plasmodiasis as different species requires different chemotherapy and as well strengthen the need for cytochemical stains in the diagnosis.

MATERIALS AND METHODS

Study Area

The study was conducted among patients attending Nigerian Air Force Hospital Makurdi, Benue State. Makurdi is the capital of Benue state, Nigeria. The city is located in the Middle Belt along the Benue River. It is located 7.73 latitude and 8.52 longitudes and it is situated at elevation 104 meters above sea level. Makurdi has a population of 292,645 making it the biggest city in Benue. Inhabitants of the area are mainly the Tiv and Idoma people of Benue State. Makurdi LGA has over 380 people per km^2. The males are 49.8 percent of the total population while females constitute 50.2 per cent [10]. Makurdi doubles as the capital of the state and the headquarters of Makurdi LGA [11]. Benue State, which is located in the North Central region of Nigeria, has a total population of 4,253,641 in 2006 census, with an average population density of 99 persons per km^2 . This makes Benue the 9th most populous state in Nigeria. However, the distribution of the population according to Local government areas shows marked duality [11].

The State lies within the lower river Benue trough in the middle belt region of Nigeria. Its geographic coordinates are longitude 7° 47' and 10° 0' East. Latitude 6° 25' and 8° 8' North; and shares boundaries with five other states namely: Nasarawa State to the north, Taraba State to the east, Cross-River State to the south, Enugu State to the south-west and Kogi State to the west. The state also shares a common boundary with the Republic of Cameroon on the south-east. Benue occupies a landmass of 34,059 square kilometres [11].Benue State experiences Wet season and the Dry seasons. The rainy season lasts from April to October with annual rainfall in the range of 100-200mm. The dry season begins in November and ends in March. Temperatures fluctuate between 21 – 37 degrees Celsius in the year. The south-eastern part of the state adjoining the Obudu-Cameroun mountain range, however, has a cooler climate similar to that of Plateau State [11].

Study population / Sampling

A total of two hundred (200) subjects were selected randomly and will be used for this study.

Specimen Collection

Blood samples were collected from subjects using standard venipunture technique. Obtained specimens were placed into EDTA anticoagulant tube used for the test.

Test Method

Blood samples were tested by microscopic examination after preparing thick and thin blood films from the collected specimens and staining with Giemsa staining method. Thick films were used for the detection of plasmodium parasites while thin films were used for their identification. Procedure for preparation of thick and thin blood films was carried out as described by [12], while the staining procedure was carried out as described by [13].

Procedure used for Preparation of thick Blood film

A drop of fresh blood was placed at the centre of a clean slide. It was mixed in circular motion using a spreader over an area of about 2cm in diameter. The slides was laid flat and the film was allowed to dry thoroughly (at least 30 minutes), protecting it from insects

Procedure used for Staining of the thin blood film

The dried film was placed on the staining rack. It was stained with 1:10 diluted Giemsa stain for 30 minutes. It was washed with buffered distilled water of pH 7.0. The back of the stained slide was cleaned with absorbent cotton wool. The stained thick blood film was allowed to air dry. The film was examined using x100 microscope objective for the presence of the parasites

Procedure used for producing thin blood films

A small drop of well mixed blood (about 2mm in diameter) was placed at ¾ end of the slide (about 1cm from the end of the slide).The slide was placed on a flat surface and it was held down firmly at the opposite end with the thumb and the forefinger. The spreader was quickly placed just in front of the drop of blood at 45^0 angle. It was drawn back slightly to touch the drop of blood and to allow the blood flow along the contact line. The spreader was pushed forward smoothly and rapidly, maintaining the contact between the slide and the spreader. A thin film of about 3 - 4 Cm long was produced slightly thicker at the base or head and thin at the tail end without ragged tails. The produced film was allowed to air dry

Staining Procedure used for Thin Films

The air-dried film was placed in absolute methanol by dipping the film briefly (two dips) in a Coplin jar containing absolute methanol.It was removed and allowed to air dry.It was stained with 1:10 diluted Giemsa stain for 30 minutes. It was washed by briefly dipping the slide in and out of a Coplin jar of buffered water. The stained film was allowed to air dry in a vertical position. It was examined under the microscope using x100 objective.

RESULTS

The result obtained on the prevalence of the various plasmodium species was subjected to statistical analysis using frequency and percentage table

Table 1: Prevalence of plasmodium species among positive subjects

Specie	Frequency	Percentage
P. falciparum	188	94%
P. ovale	10	5%
P. malariae	2	1%
P. vivax	0	0%
Total	**200**	**100%**

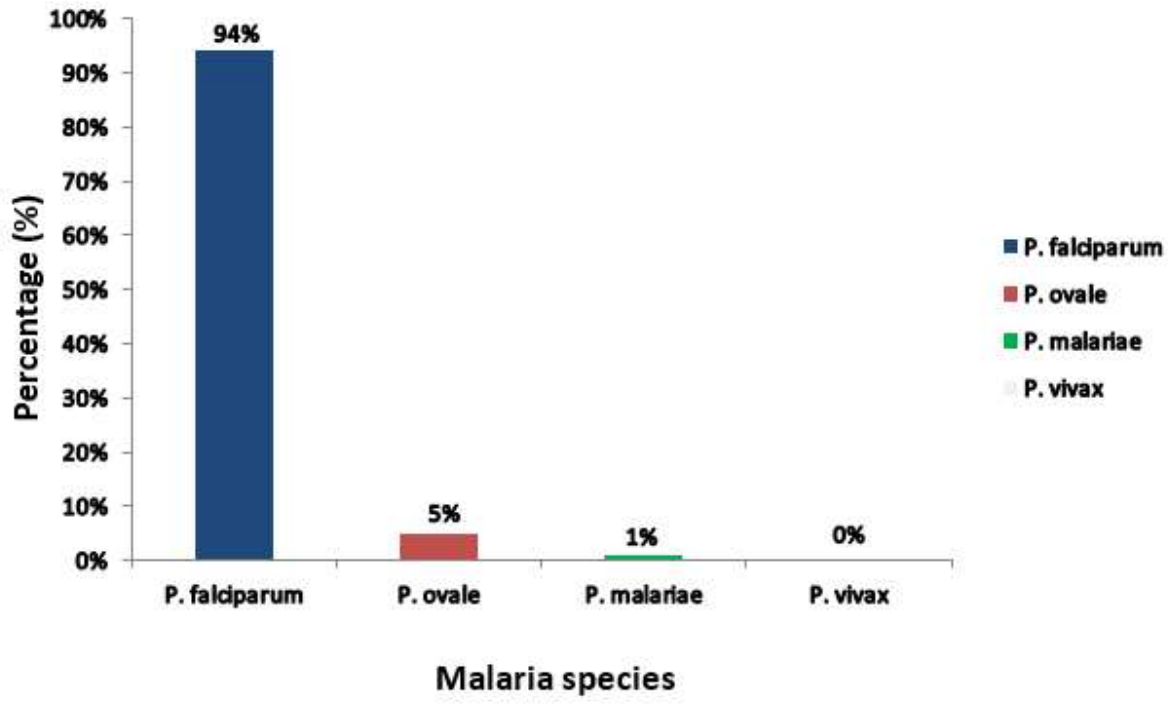

Figure 1: Prevalence of Plasmodium species among positive cases

Table 2: Prevalence of plasmodium species base on Rank

Rank	*Pf*	*Po*	*Pv*	*Pm*	Total

Officers	39(19.5%)	2(1%)	0(0%)	0(0%)	**41(20.5%)**
Soldiers	38(19%)	3(1.5%)	1(0.5%)	0(0%)	**42(21%)**
S/D of O/S	62(31%)	3(1.5%)	1(0.5%)	0(0%)	**66(33%)**
Civilians	49(24.5%)	2(1%)	0(0%)	0(0%)	**51(25.5%)**

Key: Pf = *Plasmodium falciparum*, Po = *Plasmodium ovale*, Pm = *Plasmodium malariae*, Pv = *Plasmodium vivax*, S/D of O/S = Sons and daughters of officers/Soldiers

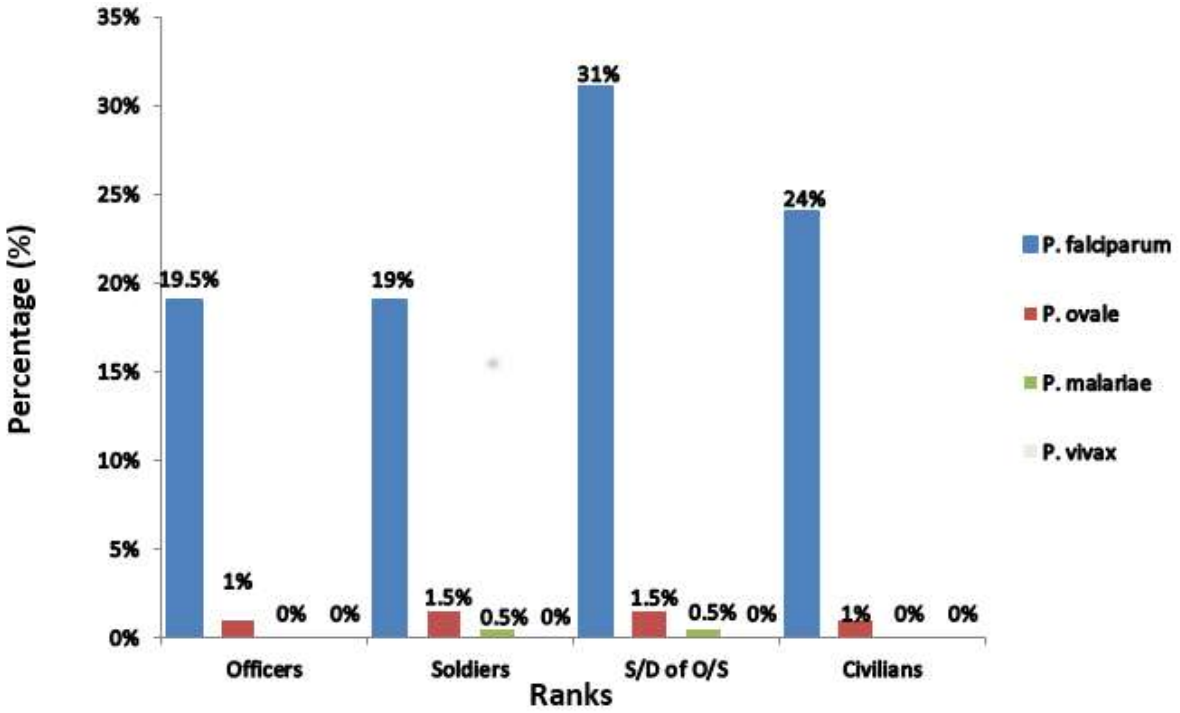

Figure 2: Prevalence of Plasmodium parasites base on ranks

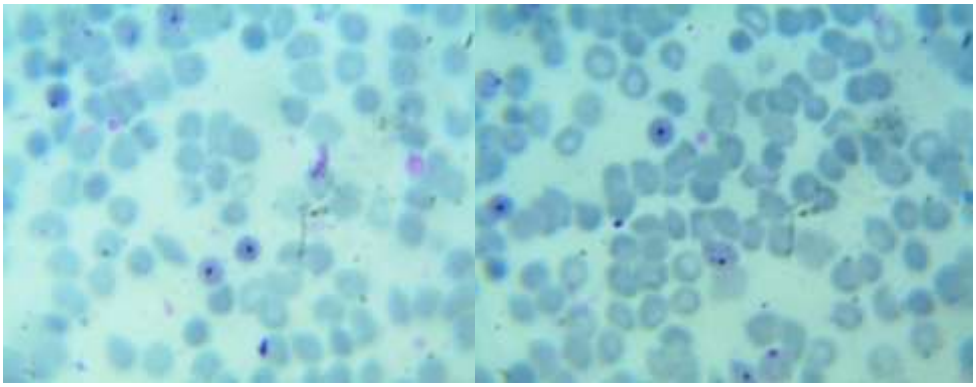

Plate 1: Photomicrograph of cytochemical reaction of *plasmodium ovale* in thin blood smear of giemsa stain

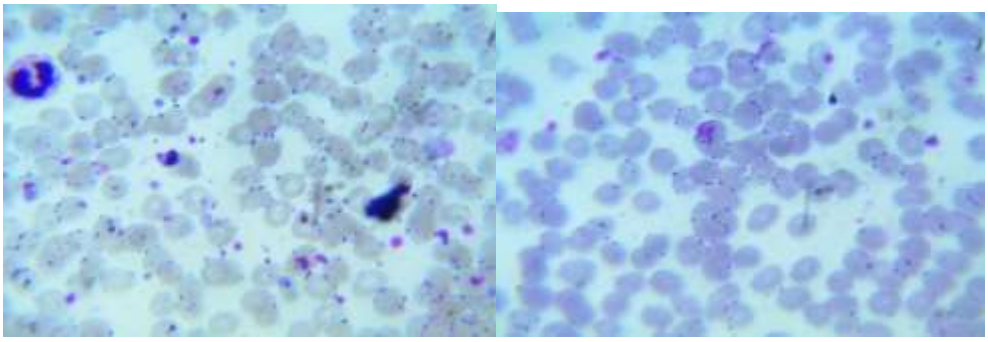

Plate 2: Photomicrograph of cytochemical reaction of *plasmodium malariae* in thin blood smear of giemsa stain.

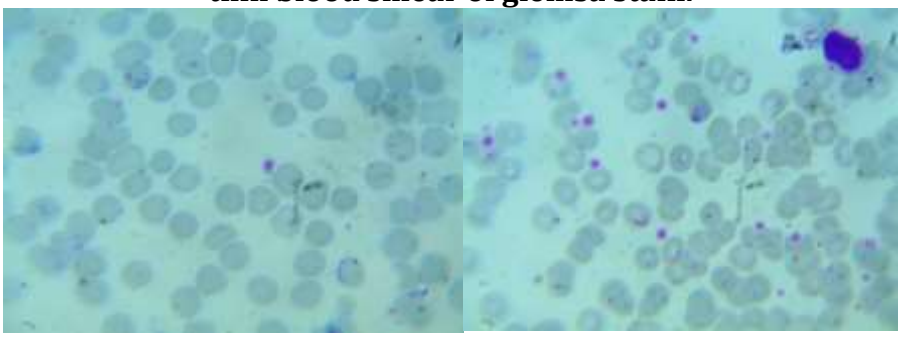

Plate 3: Photomicrograph of cytochemical reaction of *plasmodium ovale and plasmodium falciparium* in thin blood smear of giemsa stain (mixed infection).

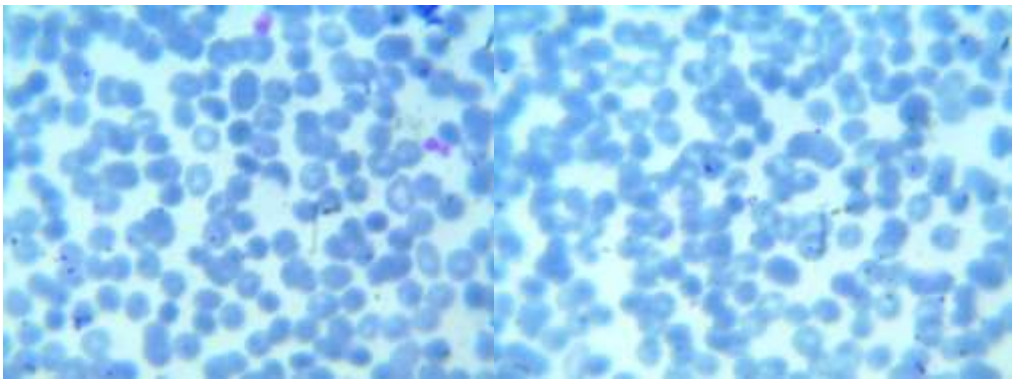

Plate 4: Photomicrograph of cytochemical reaction of *plasmodium falciparium* in thin blood smear of giemsa stain.

DISCUSSION

The data obtained from the study shown that out of the 200 plasmodiasis cases, 188 were caused by *P. falciparum* representing 94%, 10 were caused by *P. ovale* representing 5% and 2 were caused *P. malariae* representing 1%. There was neither case of *P. vivax* nor *p. knowlesi* infection representing 0%. Base on specie prevalence, *P. falciparum* recorded the highest prevalence of 94%. This result is in agreement with findings of [14] which recorded highest prevalence in *P. falciparum* infection (85.5%) than other species. Though the prevalence rate for *P. falciparum* in this study is more lower than the findings in other studies perhaps due to different geographical location

and migration life styles of soldiers whom are often on posting to different location across the country and sometimes on international operations to other African countries due to one form of political crisis or the other especially those developing or less develop African countries. On the other hand, the prevalence of *P. malariae* recorded by Sam Wabo*et al*[15], was higher (3.3%) than the prevalence in this study (1%) while that of *P. ovale* was higher (5%) in this study than that of Sam Wabo*et al* [15] which recorded *P. ovale* prevalence of (0.7%) [15]. Another similar study conducted by Kal*u et al* [16] recorded higher prevalence in falciparum infection (80%) than ovale (0%), vivax (0%) and malariae infection (2.40%). Prevalence of *P. falciparum* infection greater than the prevalence in this study was recorded by Sam Wabo et al in Ogun State where the prevalence of *P. falciparum* infection was found to be 95.6% [15]. According to Centre for Disease Control and Prevention, *P. falciparum* is the dominant plasmodium specie causing plasmodiasis in Tropical and subtropical countries such as Nigeria. This explains the reason for the higher prevalence of *falciparum* infection in the study areas. Moreso, *P. vivax* is mostly prevalent in Latin America and Asia explaining it's zero percent prevalence while *P. malariae* is prevalent worldwide and *P. ovale* is prevalent in Africa and Pacific Island. This explains their least prevalence compared to *P. falciparum* [17]. *P. vivax* infection is dependent on the Duffy blood group antigens on the erythrocytes surface which is rare in the African population. This explains it zero prevalence in the study area [15].

Infection prevalence was also studied based on ranks. The ranks included the officers, soldiers, civilians and sons and daughters of officers and soldiers. In the officers rank, 41 infected subjects were recorded representing 21.5%. Out of the 41 infected subjects, 39 were suffering from *P. falciparum* infection representing 19.5%, 2 were suffering from *P. ovale* infection representing 1%, while non was suffering from *P. vivax* and *P. malariae* infection representing 0%. In the soldier rank, 42 infected subjects were recorded representing 21%. Out of these 42 infected subjects, 38 were infected with *P. falciparum* representing 19%, 3 were infected with *P. ovale* representing 1.5%, 1 was infected with *P. malariae* representing 0.5%. Data obtained also showed that sons and daughters of officers and soldiers recorded 66 subjects out of the total infected subjects representing 33%. Out of this 66 infected subjects, 62 were infected with *P. falciparum* representing 31%, 3 were infected with *P. ovale* representing 1.5%, 1 was infected with

P. malariae representing 0.5% while non was infected with *P. vivax* representing 0%. The result also showed that civilians recorded 51 infected subjects out of the total infected subjects representing 25.5%. Out of these 51 infected subjects, 49 were infected with *P. falciparum* representing 24.5%, 2 were infected with *P. ovale* representing 1% while non was infected with *P. vivax* and *P. malariae* representing 0%. The prevalence of infection base on rank revealed that sons and daughters of soldiers (<18years) recorded the most infected subjects (33%) followed by civilians (25.5%) while officers recorded the least prevalence of *plasmodiasis* (20.5%) followed by soldiers (21%). This result is in agreement with findings of Nas et al., which recorded higher prevalence of *plasmodiasis* among subjects in the younger age groups [18]. This finding is also consistent with findings of Kalu*et al* [16] which recorded higher prevalence in the younger age group and least prevalence in the older age group [16]. The highest prevalence recorded among the younger age group could be attributed to absence of adaptive immune response against plasmodium parasites in the younger age group. On the other hand, the least prevalence of infection seen in officers who were subjects in the older age group can be attributed to the development of adaptive immunity against the parasites due to continuous exposure to the parasites. Sons and daughters of soldiers recording the highest prevalence of infection also recorded the highest burden of *P. falciparum* infection followed by civilians. *P. ovale* infection was most prevalent among soldiers and those in the younger age group (sons and daughters of soldiers and officers). The two ranks also recorded the only *P. malariae* cases in the study area. This may be because they are the likely groups to get exposed to the infection due to their nature of work and habit, in addition the cytochemical reaction of giemsa dye has demonstrated all the cytomorphological features of the plasmodium species and its microscopy remains the gold standard in the diagnosis of malaria, Leishman stain has also been reported to give a very good reaction, meanwhile giemsa stain have been chosen for its better optical differentiation, and its ability to demonstrate other blood or tissue parasites. Meanwhile the kites used for this study only detect *plasmodium* species and other species without speciation, that's why cytochemical reactions of the species were of significant in malaria diagnosis.

CONCLUSION

Base on the findings from the study, it was established that *P. falciparum* plasmodiasis is the most prevalent infection in the study area with a prevalence of 94%. Infection rate was higher in subjects less than 18 years old (sons and daughters of officers and soldiers) (33%). Despite the current progress of *plasmodiasis* control efforts in Nigeria, where most states are not *plasmodiasis* free and the total number of cases has been steadily increasing, Nigeria is not yet on its way to achieving those original eradication goals. A key aspect of future research in Nigeria should therefore focus on understanding treatment-seeking behavior, barriers to accessing health services among febrile persons, and quantifying patterns of *plasmodiasis* transmission. Meanwhile drastic effort to curb *plasmodiasis* must include the use of active insecticide to fumigate the entire Africa with the aid of helicopter; some household insecticide has shown different degrees of resistance as a result of less dilution and ineffectiveness. *Permethrin* 0.4% and *tetramethrin* 0.4% have shown good insecticidal sensitivity against mosquito in Nigeria and less effect has also been observed upon usage of 0.2% of both *permethrin* and *tetramethrin*. The next to fumigation is vaccine production and administration; it is necessary that the species of mosquito across Africa are researched on and possible vaccines produced to totally eradicate malaria.

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COMPETING INTEREST

Authors have declared that no competing interest

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