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Incidence of malaria parasites in symptomatic patients attending 44 Nigerian Army Reference Hospital (NARH) Kaduna state

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ABSTRACT

Background: Malaria is a major public health problem and cause of much suffering and premature death in the poorer areas of tropical Africa, Asia and Latin America. This study investigated the incidence of malaria parasites among symptomatic patients in 44 Nigerian Army Reference Hospital, Kaduna State. **Method:** 100 blood samples were collected from patients attending 44NARHK. A thick and thin film were made using these samples and then stained using the 10% giemsa solution and then viewed under the microscope. Questionnaires were distributed to patients in order to identify the risk factors that makes the *plasmodium specie* thrive in the human body. **Results:** A total number of 100 blood samples were collected from patients who showed symptoms of malaria and was tested using the microscopic examination of blood. Of the 100 samples that were examined, 23 were positive giving an incidence rate of 23%. The incidence of malaria parasites among the patients was significantly associated with gender; females had the highest incidence of 56.5%, the age group of 19-30years had an incidence rate of 43% and the genotype AA also had the highest incidence of 73.9%. **Conclusion:** This study has demonstrated the incidence of malaria parasite in patient who showed symptoms like fever/headache, also the geographical location and environmental condition of patients contributes to breeding of the malaria vector. Hence, the study recommends that more work be done on the vector and the parasites.

Introduction

Malaria is one of the most serious tropical diseases, accounting for the majority of hospitalizations and deaths among children and adults [1,2]. Malaria is an infectious disease that continues to be associated with considerable morbidity, mortality, significant social and economic impact on developing societies [3,4]. The World Health Organization reported that there were an estimated 246 million malaria cases distributed among 3.3 billion people at risk in 2006, causing at least a million deaths. Malaria is typically diagnosed by the microscopic examination of blood using blood films, or with antigen-based rapid diagnostic

tests [5]. Methods that use polymerase chain reaction to detect the parasites' DNA have been developed, but are not widely used in areas where malaria is common due to their cost and complexity [6]. The diseases are most commonly transmitted by an infected female anopheles' mosquito. The mosquito bites introduce the parasites from the mosquito's saliva into a person's blood [7]. The parasites travel to the liver where they mature and reproduce. There are five different species of the malaria parasites (*Plasmodium*) that can infect and be spread by humans to other human beings [5]. Most deaths are caused by *Plasmodium falciparum*

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because malaria caused by *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae* are mostly mild cases. The species of *P. knowlesi* rarely cause disease in humans and the exact mode of transmission remains unknown.

Plasmodium presents a public health problem and cause suffering and premature death in tropical countries, because of their ample rainfall, and long periods of warmth which favors both mosquito breeding and human infection [8].

In many endemic areas, malaria increases and it's difficult to control because of the resistance of the parasites to antimalarial drugs and the failure of vector control measures: at present, about two billion populations live in malarious zone. Every year, 200-300 million people die due to the disease, most of the victims are children and pregnant women. *Plasmodium malariae* is widely distributed but less common, *Plasmodium ovale* is rare but tends to replace *Plasmodium vivax* in West Africa where it has been linked with lack of Duffy blood group [9].

Malaria causes symptoms that include fever, fatigue, vomiting, and headaches. It can also cause yellow skin, seizures, coma, or death in severe cases [5]. Symptoms usually begin 10-15 days after being bitten. People may get recurrences of the disease months later if they are not appropriately managed. Reinfection usually causes lesser symptoms in persons who have recently survived an infection. If the person is not exposed to malaria for months or years, the partial resistance diminishes [5].

The goal of this study was to illustrate the incidence of malaria parasites in patients who visited the hospital for a checkup and displayed symptoms. The objectives were to determine the population at risk, identify the most frequent *Plasmodium* species implicated in the illness, and learn about the risk factors that could lead to infection.

Materials and Methods

Collection of samples

The skin was cleaned using a swab moistened with mentholated spirit and allowed to dry completely. A tourniquet was used to firmly tighten the patient's upper hand, and a new syringe and needle were used to penetrate the vein with caution, the blood was carefully collected to avoid damage to the vein tract. The tourniquet is loosed and the needle was withdrawn immediately from the vein. A dry cotton

wool was placed on the inserted spot and the blood was dispensed into an EDTA container and gently mixed to avoid clotting [10].

Giemsa stain preparation

Giemsa powder (3.8g), 250ml glycerol, and 250ml methanol were measured and used to make a 500ml Giemsa working solution [11]. The methanol was measured and added to the pre-weighed powder in a dry cylinder, and thoroughly mixed. The same cylinder was used to measure the glycerol and was added to the stain and mixed well. The bottle of stain was placed in a water bath at 50-60^oc for up to two hours to help the stain dissolve well. It was mixed at intervals and kept in a bottle of hard glass with a close filtering glass stopper to protect it from sunlight.

Dilution of Giemsa stain: the stock solution was diluted by mixing the appropriate amount of it with distilled water. The dilution is of two types, 3% solution for 30 minutes of staining, and 10% solution for 10 minutes of staining. The 10% solution for 10 minutes of staining was used in this study; 45ml of buffered water, pH 7.1-7.2, was measured in a measuring cylinder. 5ml Giemsa stain was added and gently mixed [12]. This technique, known as the fast test dilution method, was applied in this research.

Making of the thick and thin films

Thick smear was made by touching the drop of blood with a glass slide held above the blood and then reversing the slide. The blood was evenly spread with the corner of another slide to make a square or circular patch of a moderate thickness that will just allow one to read through it. While drying, the slide was positioned horizontal and shielded from dust and flies. Thin film is made by placing a drop of blood (smaller than the thick film) on the slide. The smooth edge of another clean glass slide or cover slip is applied at an angle of 45 degrees to the drop of blood, touching it and spreading it along the edge; the spreader is pushed forward at the same angle [13]. A properly made thin film should consist of an unbroken layer of single red blood cells with a structure that looks like a "tongue" not touching the edge of the slide.

Staining thick and thin films

The thick films were dried and the thin films were fixed in methanol solution for one to two minutes. The freshly prepared staining solution was used to stain both thick and thin films (i.e. after mixing 5ml of Giemsa stock solution with 45ml of buffer solution). The slides were kept vertically over a

glass rod and the stains were poured on them covering the whole slide and allowed to act for ten minutes [14]. The slides were gently rinsed with buffered solutions and the slides were then allowed to stand upright to dry before microscopic examination

Microscopic examination of films

A drop of oil immersion was placed on the films and viewed under the microscope at x100 objective lens. The presence of malaria parasites was observed on the thick films and the different species of the thin films and recorded [15].

Use of structured questionnaires

Accurately structured questionnaires were used to determine the patient socio-demographic factors like age, gender and genotype. These questionnaires were also used to identify the certain risk factors associated with having the parasite.

Results

A total number of 100 patients were examined. Out of these, samples from 23 patients indicated a positive results showing the presence of three different stages of malaria parasites. Below is a pie chart showing the incidence rate of examined blood samples of patient.

Incidence of malaria parasite of examined patients in relation to age: Patients with the age range of 19-30 years had the highest incidence while those with the age range 51 and above had the lowest. Out of the 23 positive results obtained, 43.4% were from the age range 19-30 years, 13.04% were from both age range 0-18 and 31-50 years, 4.3% were from the age range 51 and above and 26.08% were from those who were adults.

Incidence of malaria parasite of examined patients in relation to gender: It was determined that the female patients had the higher incidence of 23.6% than the male who had an incidence of 22.2%. Out of the 23 positive result, the percentage of female positive was 56.5% while that of male was 43.4%

Incidence of malaria parasite of examined patients in relation to genotype:

Patients with genotype AA had the highest incidence while those with SS had zero incidence. Out of the few patients with AS genotype that were examined, a 28.5% incidence (the highest) was observed. Out of the 23 positive results obtained, 73.9% were from patient with genotype AA, while 26.1% were from patients with genotype SS.

Incidence of malaria parasite in relation to different species:

The incidence of malaria parasite in examined patient attending 44 Nigerian Army Reference Hospital was recorded to be 23%. Out of this, the different species of *Plasmodium* were also noted. The incidence of patient with *Plasmodium malariae* had the highest incidence rate of 52.17%, while patient with *Plasmodium vivax* had the lowest. **Figure 2** below is a bar chart showing the incidence showing the various *Plasmodium* species.

Incidence of malaria parasite in relation to sewage disposal methods of patients:

Patients with good sewage disposal system had the highest incidence while those with a fair one had zero incidence. Of the few patients with poor sewage disposal system that were examined, a 12.1% incidence (highest) was obtained. Out of the 23% positive result obtained, 21.7% were from both those with a good and poor sewage disposal system. **Table 1** shows the incidence rate in relation to adequate the sewage disposal system is in the home of the patient from the structured questionnaires used.

Incidence of malaria parasite in relation to usage of insecticide treated net (ITN):

Patient that used mosquito net had a high incidence of 30.5% while those that didn't had an incidence of 4.6%. out of the 23 positive results obtained, 47.8% were from patient that used mosquito net, while 13.0% were from those that didn't. **Table 2** shows the incidence rate in relation to patient response to the use of ITNs from the structured questionnaire.

Table 1.The incidence rate in relation to adequate the sewage disposal system is in the home of the patient.

Factors	Number	No. of Positive	%Infected
Sewage disposal			
Good	47	5	10.6%
Fair	12	0	0%
Poor	41	5	12.1%

Table 2. The incidence rate in relation to patient response to the use of ITNs from the structured questionnaire.

Factors	Number Tested	No. of Positive	%Infected
Use of ITNs			
Yes	36	11	30.5%
No	64	3	4.6%

Key; ITNs: Insecticide Treated Nets

Figure 1. A pie chart showing the incidence rate determined

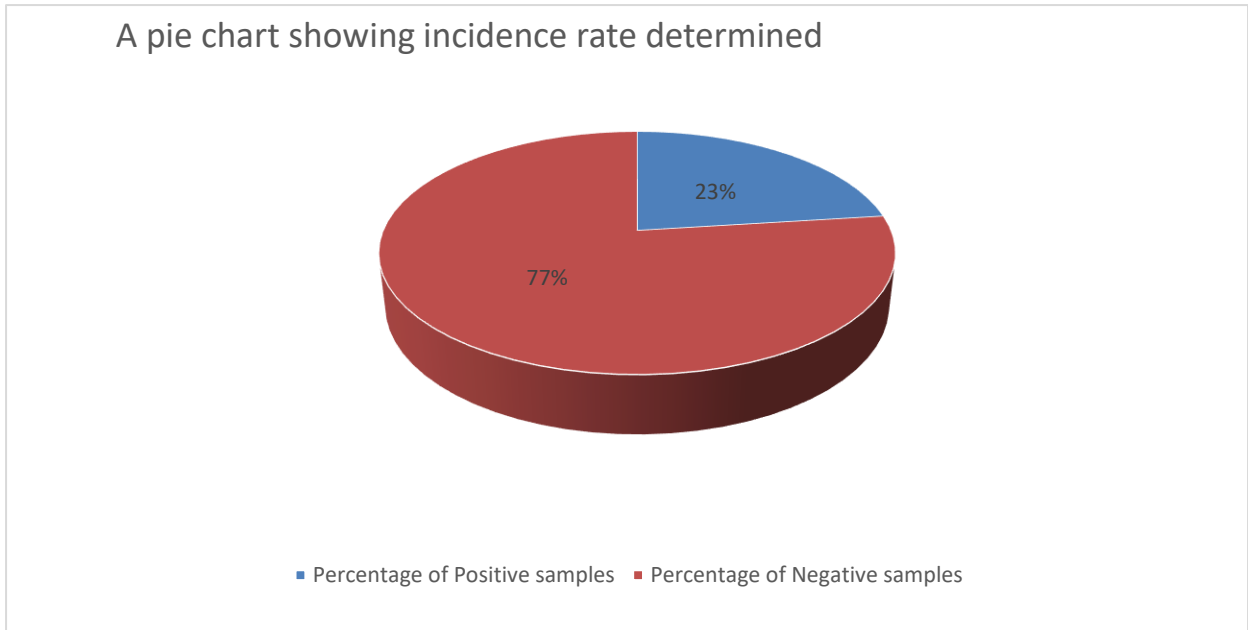
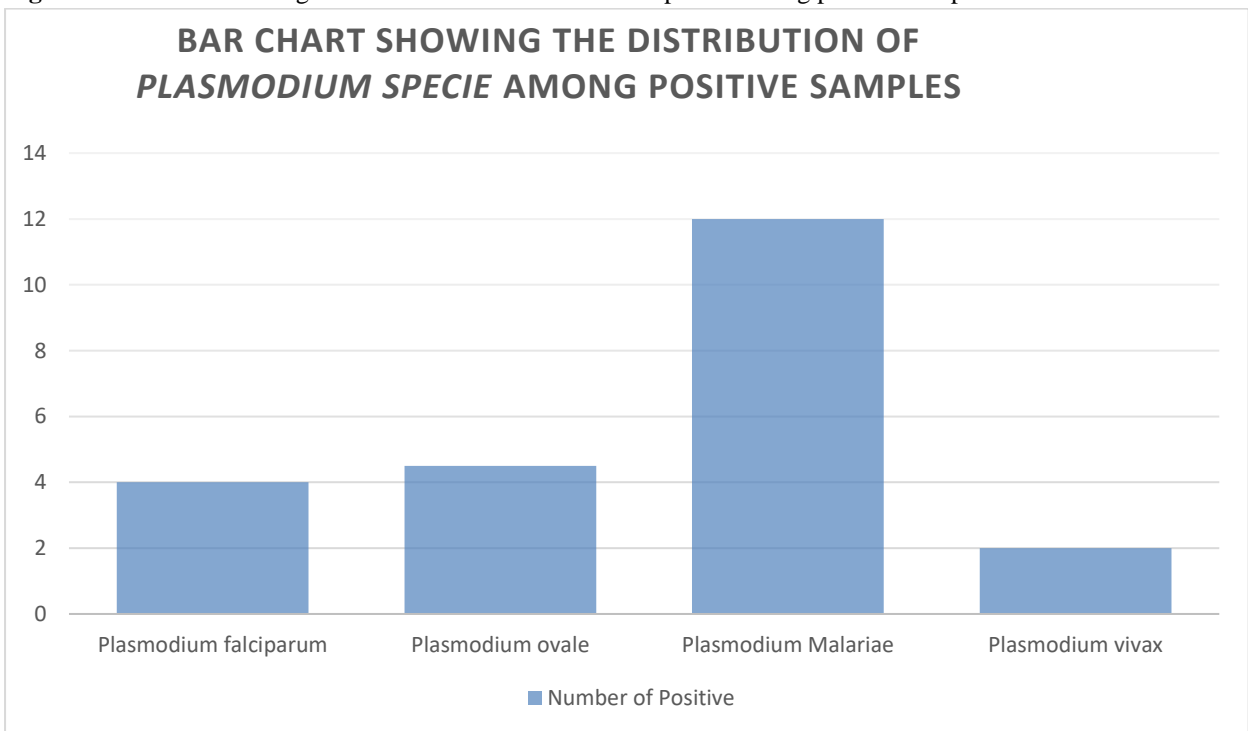


Figure 2. Bar chart showing the distribution of *Plasmodium* species among positive sample.



Plates 1,2,3 are also pictures showing how various specie look under the microscope.



Plate 1: Trophozoite of *Plasmodium malariae* **Plate 2:** Gametocytes of *Plasmodium vivax* **Plate 3:** Trophozoite of *Plasmodium ovale*

Discussion

In this study, a total of 100 samples were collected and examined for malaria parasites. The study shows that 23% of the patients in the study area were positive for malaria parasites. The observed incidence is in line with the findings of [16] who reported a high incidence rate of 42.8% among some patients in Owerri, Nigeria. Three stages (Trophozoites, Schizonts and Gametocyte) of the four species of *Plasmodium* were observed under the microscope. The result is in contrast with the result recorded in south east Nigeria showing a prevalence of 76.8% for *Plasmodium falciparum* [17]. This could be due to the geographical region where the study was taken and awareness of the public on how to prevent malaria. A previous similar study result in southwest Nigeria is in agreement, with a high prevalence of 54% for *Plasmodium malariae* [18].

Out of the 23 positive samples gotten, 56.5% were from females compared to males although no significant difference was observed statistically. This agreed with the findings of [19]. The age group 51 years and above had the lowest incidence of malaria parasites (4.3%) while the 19-30 years' group had the highest incidence of 43.40%- this may be due to the depression of immunity level as a result of stress for the adults age range and the lower age group may have a higher level of immunity because of previous exposure in their childhood. Meanwhile there is yet to be any recorded literature review that ascribes susceptibility to malaria parasites to any age group, except the immunocompromised, pregnant women and children [20].

Patients with genotype AA also had a high incidence of 73.9% than patients with genotype AS or SS. Malaria parasites have a high rate of oxygen consumption and absorbs huge amounts of hemoglobin during the peripheral blood stage of

reproduction, according to a previous study. The AA blood contains a large amount of hemoglobin in its blood, this makes the AA blood more conducive and malaria parasites thrive better in it [21].

Plasmodium malariae had an incidence of 43.4% among the 23 positive samples gotten when compared to other species of *Plasmodium*. This could be because *P.malariae* does not have a hypnozoites stage, but patients infected with this species of *plasmodium* may have a prolonged, asymptomatic erythrocytic infection that becomes symptomatic years after leaving the endemic areas. Because *P. malariae* infection is typically relatively mild, *P. knowlesi* infection should be suspected in persons residing or travelling in Southeast Asia or South America who are severely ill and have microscopic evidence of *P. malariae* infection [21].

Conclusion

In conclusion, the geographical region where the patients examined lived and its high prevalence is as a result of the historical background of the species, the agricultural practice and poor environmental sanitation which provides a suitable breeding environment for the parasite vector.

This study shows that the incidence of malaria parasites among examined patients in the study area is 23%, the most dominant *plasmodium* specie is *P. malariae* (43.4%) and the most susceptible gender, age range and genotype were females, age group 19-30 years and patients with genotype AA respectively.

It can be inferred that one in every three patients with the malaria parasites belong to one if not all these groups. In summary, there is a high incidence among females who were adolescents with the genotype AA in the study site during the course of this study.

Competing interest

Author declares that no competing interest exists.

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