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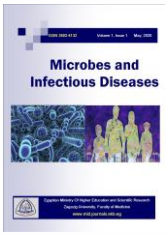

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Original article

Prevalence of *Plasmodium vivax* species in Elhajyosife area in Shergalnile locality in Khartoum State

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BF: Blood film
EDTA: Ethylenediaminetetraacetic acid
PCR: Polymerase chain reaction
RDT: Rapid Diagnostic Test
WHO: World Health Organization

ABSTRACT

Background: *Plasmodium vivax (P. vivax)* malaria has been recognized as an important cause of morbidity in several African countries. The prevalence was previously estimated as 12.5% in Khartoum. These estimates are observed to be rising and spreading continuously. **Objectives:** The present study was undertaken to investigate the situation of distribution of *P. vivax* malaria in Elhajyousiff area in Khartoum State-Sudan. **Methods:** Cross-sectional malaria surveys carried out in Elhajyousiff area (Khartoum State) during the period of August 2021 to September 2021. Atotal number of 250 blood samples were examined by microscopical examination. The samples were collected from different centers in Elhajyousiff area (Albanjadeed Hospital, Almaygoma Health Center and Alsheeda Nada Health Center). All samples were examined by direct microscopy for plasmodium species using blood film (Thick and thin). **Resuls:** From 250 samples there were 241(96.4%) samples negative, 5(2.0%) samples were positive for *Plasmodium falciparum (P.falciparum)* and 4(1.6%) samples were positive for *P.vivax*. The *P. falciparum* showed as more common species in area. **Conclusions:** Even though malaria in Shergalnile locality is still largely recognized to *P. falciparum*, *P. vivax* has been growing with worrying proportions and spreading to new areas. The emergence and marked increase of *P.vivax* poses new challenges to malaria treatment and control in Elhajyousiff area.

Introduction

Malaria is a serious vector-borne tropical disease that remains one of the primary reasons of death in several developing countries [1].

The number of human infections continues to increase in countries where the disease is endemic as well as in regions where the disease is not endemic [2]. The distribution and severity of malaria depends on the interaction of a number of factors. These include the size of the infective dose of sporozoites, nutritional status of the host, level of acquired immunity, host genetic factors, parasite growth rate, drug resistance status, socioeconomic conditions, standard of health care and education

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[3]. In areas with lower transmission (eg. Khartoum State), infections are less frequent and a larger proportion of the older children and adults have no protective immunity. In such areas, malaria can be found in all age groups.

In Sudan almost, 75% of population is at risk of developing malaria. Malaria transmission is unstable putting the whole country under the risk of malaria epidemic. The possibility of epidemic increased with heavy rains and floods [4]. Much effort and progress to control and eliminate malaria has been made by the World Health Organization (WHO), affected countries as well as many other cooperative partners [5]. Malaria occurs in poor regions and poor countries, and its presence has a strong negative correlation with economic growth in families, communities, and nations. Where *Plasmodium vivax (P.vivax)* prevails, people of all ages, adults and children alike, experience malarial disease. Moreover, moderate though the effects of *P.vivax* malaria may be now compared with former times, the global toll imposed by this parasite is great in relation to the standards of health and well-being that could otherwise be expected in today's world, even in many of the poorest nations. Furthermore, it has been shown that *P.vivax* is able to infect even Duffy-negative African patients [6]. This previous old paradigm of *P.vivax* as "benign tertian malaria" has been challenged recently by recent reports and documentation of severe *P.vivax* disease and even deaths due to *P.vivax* mono-infections [7]. Mixed infections with asexual blood forms of *P. falciparum* and *P.vivax* are well described but relatively uncommon compared to single species infections. Shute (1951) described the frequency of mixed infections as less than 1% among the hundreds of British troops he examined in southern Italy during 1943 [8]. Traditionally *P.falciparum* has been thought to inhibit the parasitaemia of *P.vivax* [9]. In contrast, there are several lines of evidence suggesting that *P.vivax* may have a suppressive effect on *P.falciparum*. James, in his classic review of studies of induced malaria was impressed that *P.vivax* was the predominant species [10]. There are several technique to diagnosis of malaria worldwide. The routine lab usually uses two techniques thick, thin blood film and immunochromatographic diagnostic test (ICT) to diagnose malaria for diagnosis of malaria.

Because of it is medical significance, the technique used in diagnosis of malaria should be very specific and sensitive. Also, *P.vivax* malaria was confirmed by polymerase chain reaction (PCR) [11]. The vast majority of these reports on severe *P.vivax* malaria are from south East Asia and India, there are few published data on severe *P.vivax* from Africa [12].

The study aimed to investigate the situation of distribution of *P. vivax* malaria in Elhajyousiff , Khartoum State, Sudan.

Materials and Methods

Study setting

This study was carried out in the Elhajyousiff area in Shergalnile locality in Khartoum State. That was from the basis of a prospective, enhanced facility-based surveillance of fever cases (defined as a history of fever during current illness history and/or an axillary temperature >= 37.5 ^{o}C.

Study design and population

This is descriptive cross sectional, quantitative approach study. The surveys were carried out in August 2021 to September 2021.

Sample size

The study was carried out on 250 suspected individuals by using Mendenhall law:

The classical statistical method for determining sample size based on an unknown proportion of markers was used at 95 percent confidence level and 10 percent precision. Since there is no known figure for the prevalence of malaria, a prevalence rate of 50 percent was used to calculate the sample size using the formula below as described by Mendenhall *et al*. (1981) [13] .

$N = Z^2 P (100 – P)/ d^2$

Where n = sample size

P = prevalence rate

Z = 1.96 at $\alpha = 0.05$ (α = desired confidence level)

d = desired width of confidence (precision)

Q = 100-P

Data collection

Medical doctors filled a clinical form to collect demographic data and signs and symptoms of malaria; (fever, chills, sweating, headache, myalgia, arthralgia, abdominal pain, nausea, vomiting, dizziness, cough, dyspnea, and diarrhea).

The recruited patients filled a questionnaire with a question about their residence status.

Collection of blood samples

A venous EDTA-blood sample was collected by venipuncture from each individual. After thin and thick blood smears were prepared.

Preparation of thick film

After collection of blood on a clean and grease free glass slide, thick film was made by spreading one drop of blood with a spreader evenly on an area about 15×15 mm in diameter. Care was taken to avoid rouleaux formation. Then, the slide was labeled properly and allowed to air-dry by keeping the slide on horizontal position. Precaution was taken during spreading and drying [14].

Preparation of thin film

After collection of one drop of blood on a clean grease free slide, thin film was made by spreading the blood using a smooth edged slide or spreader at an angle of 45º from the horizontal plane. A well-prepared thin blood film was judged by having a smooth tail end and free of vertical lines and holes. The slide was then labeled properly and allowed to air-dry [14]. Absolute methanol or ethanol was used to fix the thin film. Following steps were taken for fixing the thin film as described by **Cheeshbrough** [14]:

The slide was placed horizontally on a staining rack. A small drop of absolute methanol or ethanol was applied to the thin film. Then the slide was allowed to fix for 1-2 minutes.

Staining of the films

The slide was first placed on a staining rack. Then 10% Giemsa stain having a pH of 7.2 was poured gently on the fixed thin film or de-hemoglobinized thick film until the slide was totally covered. Then the slide was allowed to stain for 30-45 minutes out of the sunlight. Then the stain was washed with clean water. Back of the slide was wiped and placed in a draining rack. The slide was then allowed for air dry [14].

Diagnosis by microscopy using thick and thin blood film staining procedure

The blood films were stained by freshly prepared 10% Giemsa stain for 10 min and carefully rinsed with buffered water then dried. The thick part of the blood film was examined using 100 x magnifications, and up to 100 fields were checked before considering the slide as negative. The thin film was examined for *Plasmodium* species identification. The slides were examined by two experienced microscopists at the field site and then rechecked by a more experienced laboratory technician at the Parasitology Department-Administration Lab-Khartoum state, following WHO criteria for malaria diagnosis [15].

Statistical analysis

The data collected were statistically analyzed to generate Pearson correlation coefficient (p-value) using Statistical Package for Social Sciences (IBM SPSS Statistics 21) to compare the values observed.

All information gathered via data master sheet then coded into variables both descriptive and inferential statistics involving Pearson Chi-Square test were used to present results, a *p* value of less than 0.05 was considered as statistically significant.

Ethical considerations

Ethical approval was obtained from the Federal Ministry of Health. Permission obtained from hospitals in Khartoum state, they were informed and consent from each participant was obtained prior to their participation in the study. Study data/information was used for the research purposes only. The privacy issues intentionally are considered.

Ethical clearance code number: MH-RES/06-020-09

Date: 2/7/2021

Results

Demographic results

In this study the prevalence of *P. vivax* in Elhajyousiff area was estimated. A total of 250 samples were collected from different centers including Albanjadeed Hospital (100 (40%), Almaygoma Health Center (75(30%) and Alsheeda Nada Health Center (75(30%) as shown in (**Figure 1)**.

The samples were collected from both gender (female: 141 (56%), male: 109(44%) **(Figure 2)**.

Individuals with different age group and who suspected with malaria infection were included in this study and later confirmed as malaria patient with microscopy **(Figure 3)**.

Figure 1. Distribution of samples collected according to the health centers**.**

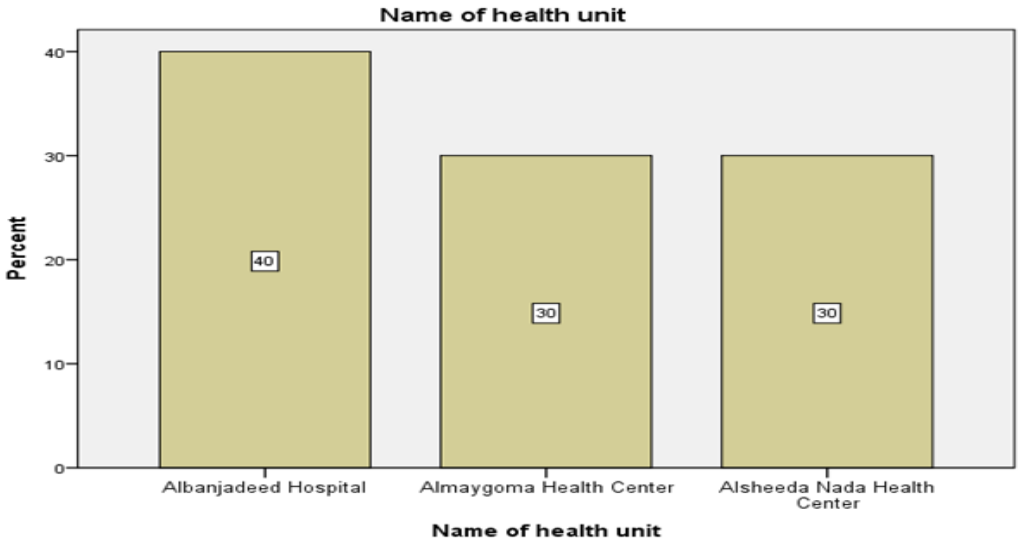
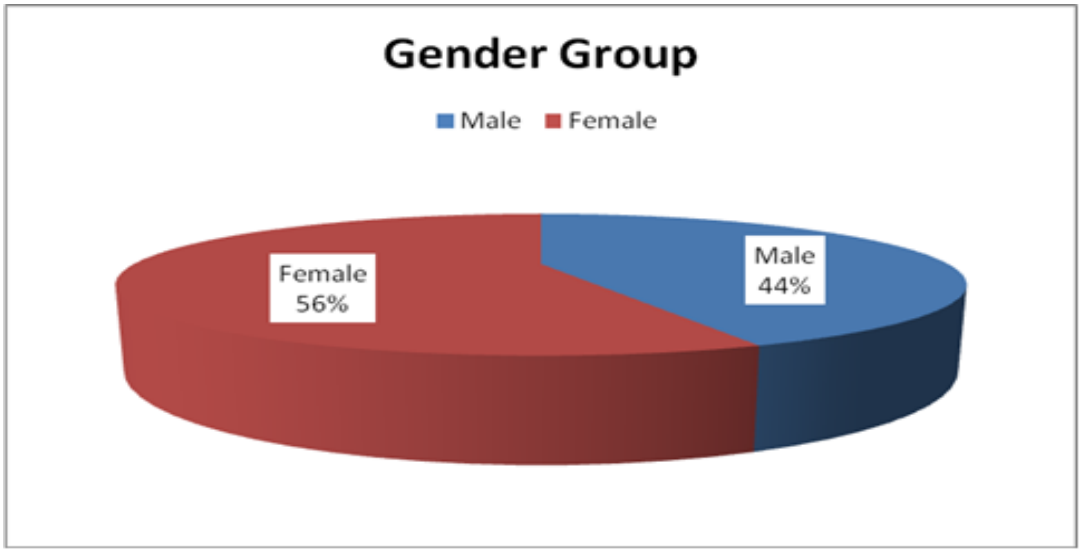
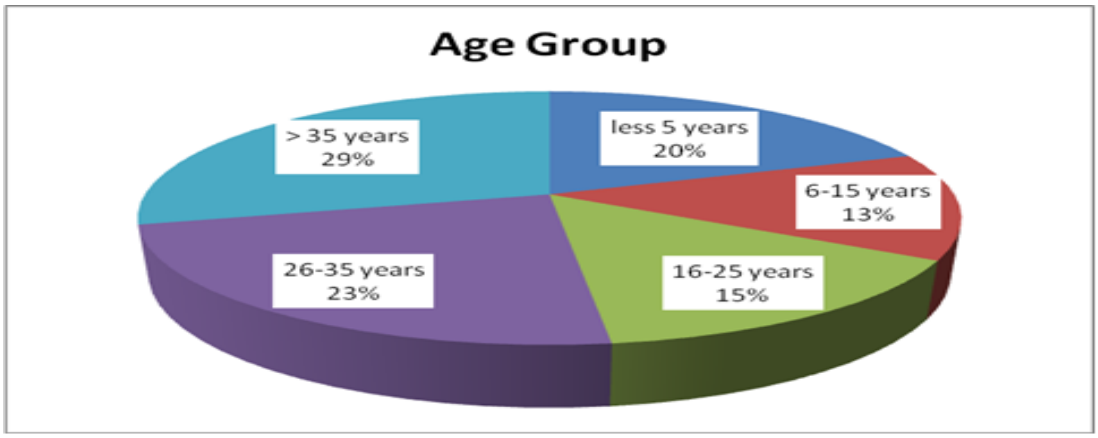


Figure 2. Distribution of study population according to gender.



This figure showed that gender males represented 44% and females represented 56% among study population.

Figure 3. Distribution of samples collected according to age group.



Microscopy was adopted as gold standard method for all the 250 samples that came positive or negative with antigen method still had same results by microscopy **(Table 1)**.

From all positive samples *P. vivax* was 4(44%) and *P. falciparum* was 5(56%) **(Table 2)**.

Each centers area showed different smear outcome, while Almaygoma represented the highest positivity rate (2%) and Alsheeda Nada represented the lowest positivity rate (0.4%) **(Table 1)**.

Table 1. The prevalence of malaria among patients according to centers.

			Result			Total
			Negative	*Pf*	*Pv*	
Name of health unit	Albanjadeed Hospital	Count	97	1	2	100
		% of Total	38.8%	0.4%	0.8%	40.0%
	Almaygoma Health Center	Count	70	4	1	75
		% of Total	28.0%	1.6%	0.4%	30.0%
	Alsheeda Nada Health Center	Count	74	0	1	75
		% of Total	29.6%	0.0%	0.4%	30.0%
Total		Count	241	5	4	250
		% of Total	96.4%	2.0%	1.6%	100.0%

Table 2. Correlations between positive results.

Parasite	*P.falciparum*	*P.vivax*	Total
	Frequency	Frequency	Frequency
	5	4	9
**. Correlation is significant at the 0.01 level (2-tailed).			

***p-value considered significant at 0.05 levels.

Table3 . Prevalence rate of species of malaria infection among patients attending hospital and health centers according to gender.

			Result			Total
			Negative	Pf	Pv	
Patient gender	Male	Count	105	2	2	109
		% of Total	42.0%	0.8%	0.8%	43.6%
	Female	Count	136	3	2	141
		% of Total	54.4%	1.2%	0.8%	56.4%
Total		Count	241	5	4	250
		% of Total	96.4%	2.0%	1.6%	100.0%

Chi-Square Tests			
	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	300.000[a]	4	.000
Likelihood Ratio	58.021	4	.000
N of Valid Cases	150		
a. 8 cells (88.9%) have expected count less than 5. The minimum expected count is .03.			

Discussion

Alhajyousif Area has been selected as the study area for the following reasons due to Inhabitants are from different tribes and racial groups, the Movement of people from eastern Sudan which contain *P. vivax* to Khartoum through Alhajyousif Area, and the presence of some recurrent malaria cases.

Plasmodium vivax has a wide geographic distribution of the human malaria parasites, at Sudan *P.falciparum* has dominant distribution especially in Khartoum state, recently *P. vivax* appears with wide distribution and the control of *P. vivax* in individuals and populations is complicated due to its ability to relapse weeks to months after initial infection [16]. Strains of *P. vivax* from different geographical areas are thought to exhibit varied relapse timings. In tropical regions strains relapse quickly (three to six weeks), whereas those in temperate regions do so more slowly (six to twelve months) [17, 18], but until now no comprehensive assessment of evidence has been conducted. This study has highlighted the prevalence of *p. vivax* in the Alhajyousif Area, the results showed the prevalence of *P. falciparum* 2%, *P. vivax* was 1.6% and the negative samples were 96.4%. The prevalence of *P.vivax* out of positive samples was 44% while *P.falciparum* was 56% and mixed sample 1.2%. (*p*.value 0.01) **(Table 2)**.

Plasmodium falciparum was the more common species in Alhajyousif Area followed by *P. vivax* and there was a statistically significant difference between them (*p*. value 0.01) **(Table 2)**.

Prevalence of *P. vivax* in Albanjadeed hospital was 0.8%, this high prevalence in this area was due to its agricultural nature and it is surrounded by Al- Selait canal which provided.

Microscopic results were initially considered as the reference standards for true positive and true negative results [9].

We concluded that the influence of *P. vivax* on the overall malaria infection rate in the Alhajyousif Area is shown to be higher and more widely distributed than previously supposed, pretension new challenges to malaria control programs and policy decision-makers. The distinct pathological features of *P.vivax* parasite strains and the high magnitude and diversity of population movement require several steps to be taken. First, specific training of medical staff on the diagnosis and treatment of *P.vivax* malaria is needed. Second, more detailed epidemiological surveys during the different seasons and entomological surveys in areas of expected high risk of infection need to be carried out. Third, the efficacy of antimalarials currently recommended for the treatment of malaria in Sudan needs to be formally tested against *P.vivax* using WHO standard drug efficacy protocols.

Ethics approval and consent to participate

Each participant was asked to sign a written ethical consent form during the interview, before the specimen was taken. The informed ethical consent form was designed and approved by the ethical committee of the Faculty of Medical Laboratory Research Board, National University-Sudan.

Consent for publication: Not applicable.

Availability of data and materials

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding: None.

Authors' contributions

SHO and SSA conceived the design and carried out the experiments. AAI and RMA and AMA and EKA obtained, analyzed and interpreted the data. MMA and HAH and RAE wrote and revised the manuscript. MAH provides financial support for all experiments. All authors read and approved the final manuscript.

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