

## Prevalence and Molecular Identification of Malaria Parasite in Displaced Camps in Khartoum State, Sudan

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### ABSTRACT

A cross sectional descriptive study was carried out from November 2008 to February 2009 among displaced camps in Khartoum state (Dar Alsalam and Jabal Awlia, are urbanizing areas) to determine the prevalence of malaria.

One hundred fifty venous blood samples were collected from symptomatic subjects from each camp. In Dar Alsalam camp, the distribution of specimens was as follows: 59 from pregnant women, 46 from adult and 45 from children under 10 years of age while in Jabal Awlia camp, 48 were from pregnant for women, 52 from adult and 50 from children under 10 years of age. Thick and thin blood films were prepared from each sample for all samples, stained with 10% Giemsa and examined microscopically for plasmodium sp. Polymerase chain reaction (PCR) was applied to 10 positive samples (5 from each camp) and 10 negative samples (5 from each camp) for the confirmation of the parasitological results and characterization of the parasite. Microscopically, the prevalence of malaria in Dar Alsalam camp was (5%, as 8 out of 150 specimens were positive) and (11%, as 16 out of 150 specimens were positive) in Jabal Awlia camp.

All the 10 selected positive samples were confirmed as *P. falciparum* by PCR technology.

The negative samples of Jabal Awlia camp were negative with PCR, while in Dar Alsalam camp, two negative blood films gave positive results for *P. falciparum* by PCR.

The study concluded that study group, socioeconomic status, education level, use of bed net and the presence of trees and water well inside or outside the houses have no significant correlation to malaria prevalence in the two camps. Moreover, the study confirmed that PCR is more sensitive than blood film in the diagnosis of malaria and that *P. falciparum* is the most dominant in these two urbanized areas.

### INTRODUCTION

Malaria is a vector-borne infectious disease caused by protozoan parasites of the genus *Plasmodium*. It is widely spread in tropical and subtropical regions, including parts of the Americas, Asia, and Africa. Each year, there are approximately 515 million cases of

malaria, killing between 1-3 million people, the majority of whom are young children in Sub-Saharan Africa, where 90% of malaria-related deaths occur. Malaria is commonly associated with poverty, but is also a cause of poverty and a major hindrance to economic development (Snow *et al*, 2005).

Severe malaria is almost exclusively caused by *P. falciparum* infection and usually arises 6-14 days after infection. Consequences of severe malaria include coma and death if untreated young children and pregnant women are especially vulnerable (Trampuz *et al*, 2003). Severe malaria can progress extremely rapidly and cause death within hours or days (Trampuz *et al*, 2003).

In Sudan malaria is endemic throughout the country with 80% of the population living in endemic-prone areas. More than 90% of cases are caused by *P. falciparum* and the primary vector is the *A. arabiensis*, 7.5 million cases and 35,000 deaths are due to malaria every year. Malaria accounted for 37.2% of the maternal deaths in Sudan at hospital level (NMCT, 2004). It also contributed by 4.8%-17.4% of all out patient clinic visit, it leads to 9.6%-36.3% of all hospital admission and results in 10%-15% of total deaths that occur at hospital level. Cases fatality rate is ranging between 0.9%-6.9 % (FMH, 2004).

The enormous economical impact of malaria is a result of the reduction in the productivity among farmers as calculated by lost working hours and days, a total of 8409 hours within 1579 days were lost as a direct result of malaria (Nur, 1993). It costs an amount of US\$5.2 per case in Gazira State (Hiatham, 2006).

The most reliable method for diagnosis of malaria is microscopic examination of stained blood films, utilizing both thick and thin smears for definitive diagnosis (Warhurst and Williams, 1996). Moreover, parasite nucleic acids are detected using polymerase chain reaction (PCR). This technique is more accurate than microscopy. However, it is expensive, and requires a specialized laboratory (Redd *et al*, 2006). However, nested PCR was found more sensitive especially in cases with low parasitemia and mixed

infections of malaria.(Stephanie *et al*, 2006).

Refugees are considered as one of the world's most critical issues that affect 47 countries. Approximately, in the continent of Africa precisely, there are about 13.5 million refugees, this means 54% of the world's total number of the refugees which is 25 million . Khartoum has been suffering from the refugees issues since the 1980s. This problem has correlated with many issues like drought, natural crisis and civil war.

The major aim of this study was to evaluate the prevalence of and to characterize malaria in displaced camps in Khartoum state (Dar Alsalam and Jabal Awlia).

## MATERIALS AND METHODS

### Study Area and Population

This study was conducted in two displaced camps in Khartoum State (Dar Alsalam and Jabal Awlia) from November 2008 to February 2009.

### Study Subjects

One hundred and fifty blood samples were randomly collected from 150 symptomatic subjects in each camp; 50 from pregnant women, 50 from children under 10 years of age and 50 from adults above 15 years.

Study subjects were given their informed consent, socio-economical and ecological data were collected, then venous blood sample were collected in ethylene diamine tetraacetic acid (EDTA).

### Microscopic examination of blood samples

From EDTA collected venous blood samples, thin and thick films were prepared, stained with Giemsa stain and were examined microscopically. Four infection intensity categories for malaria were considered as: + (1-10) parasites in entire smear, ++ (11-99) parasites in entire smear, +++ (1-10) parasites / one field, ++++ (>10) parasites/one field.

### Nested (PCR)

Nested Polymerase chain reaction (Nested PCR) was used after microscopic examination of blood film for five positive and five negative samples from each camp to confirm the results by using kit from Genekam Biotechnology AG, Germany (cat No 2267.2) to detect *P. falciparum*. DNA was isolated by using 10% chelex as follows: 100 µl of chelex was boiled in eppendorf tube for 10 minutes in a water bath then 50 µl of packed cells were added and boiled again for 10 minutes then centrifuged at 12000 rpm for 5 minutes, supernatant was transferred to a new eppendorf tube, then was centrifuged again as previous, and the supernatant which contains the free DNA was transferred to a new eppendorf tube. PCR program was run according to manufacturer instructions.

The PCR product was visualized on 1.5% agarose gel in TBE (1x) buffer stained with Ethidium Bromide. Negative and positive controls were used for quality control.

#### Statistical Analysis

The results were analyzed by SPSS Program using chi -square to find out the correlation between malaria and socio-economic status, study group, education level use of bed nets and the presence of water wells and trees inside or outside the houses.

### RESULTS

Out of the 150 blood films from Dar Alsalam camp the positive were 8 (5%) in which there was 1 moderate, 3 mild and 4 were very severe (Table 1).

Table 1: Frequency and intensity of malaria in Dar Alsalm camp

Intensity	Frequency	Percent
Negative	142	95%
(+)	3	2%
(++)	1	1%
(++++)	4	3%
Total	150	100%

Out of the 150 blood films from Jabal Awlia camp the positive were 16 (11%) in which there was 7 moderate, 8 mild and 1 was very severe (Table 2)

Table 2: Frequency and intensity of malaria in Jabal Awlia camp .

Intensity	Frequency	Percent
Negative	134	89.3%
(+)	8	5.3%
(++)	7	4.7%
(++++)	1	0.7%
Total	150	100%

There was no relation between the study groups and intensity of malaria ( $P = 0.071$  in Dar Alalam and  $P = 0.642$  in Jabal Awlia camp). There was no relation between gender and intensity of malaria ( $P = 0.007$  in Dar Alalam and  $P = 0.652$  in Jabal Awlia camp). Also, there was no relation between the education level and intensity of malaria neither in Dar

Alsalm, ( $P = 0.083$ ) nor in Jabal Awlia camp ( $P = 0.884$ ).

All the 150 study subjects from Dar Alsalam camp do not use bed nets because there were no mosquitoes. However, 146 (97%) subject from Jabal Awlia were found to use bed nets but there was no relation between malaria and use of bed nets ( $P = 0.570$ ).

### PCR results

Five positive blood films and 5 negative blood films samples from each camp were subjected to PCR (Fig 1). All the positive samples were positive by PCR and *P. falciparum* was the parasite

responsible for the disease. Regarding the 5 negative samples of Dar Alsalam, two were found positive by PCR while those negative from Jabal Awlia camp were all negative by PCR.

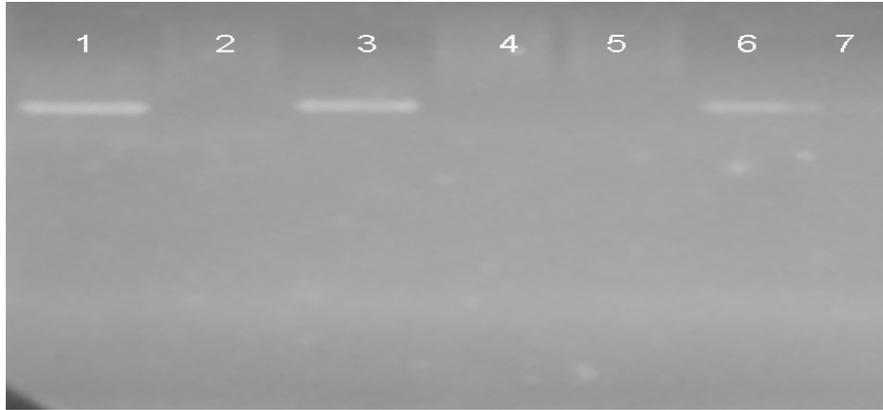


Fig. 1: Nested PCR result lanes (from left to right): lane1: +ve control, lane 2, 4, 5: -ve samples, lanes 3, 6, 7: +ve sample and lane 8: DNA marker.

### DISCUSSION

Khartoum was formerly malaria free and it was considered as hypoendemic or mesoendemic area in which malaria is unstable and epidemic outbreaks are common (El Sayed *et al.*, 2000).

The effect of twenty years of war compounded by drought and famine had a profound impact on all aspects of development in Sudan. So, the population movement from rural states (holo endemic or hyperendemic areas) has been an important feature of troubles including health problems.

In a study carried out in 2004 in Khartoum state under the aim of malaria control in an urban area, prevalence of malaria was 1% in comparison to 10 % in 1990 (NMCT; 2004).

In Dar Alsalam camp the blood films showed that 8 samples were positive for *P. falciparum*, 4 were very severe, 1 of was moderate and 3 were mild.

This area is unstable for malaria, the infection usually is related to the rainy season and there are no records for mosquito bites and any water well that

can be a site for mosquito breeding and there are no any irrigation schemes around the area.

In this study, the intensity of malaria was found very severe in adult population compared to pregnant women and under 10 year's children although these two groups are more susceptible to malaria infection due to placental malaria or slow developing immune system respectively. This may also be due to the fact that these adult population have bigger chance to get the infection from other areas considered as active foci and this is because they work in (Alkmayin) beside the River Nile in which there are mosquito breeding sites.

In Jabal Awail camp, the blood films showed that 16 samples were positive for *P. falciparum*, 1 was very severe, 7 were moderate and 8 were mild. This high prevalence rate was justified by the fact that there is a neighboring irrigation scheme (Sondos agricultural scheme) and it is considered as mosquito breeding site, despite the use of bed nets to protect themselves.

In a study carried out in (2002), Dar Alsalam camp showed the highest

infection rate of malaria followed by Jabal Awlia and Elabaraka. *P. falciparum* represents the most frequent species diagnosed from cases reported in the clinics in all camps, followed by mixed infection (*P. falciparum* and *P. malariae*) and *P. malariae* and there were very few cases of mixed infection (*P. falciparum* and *P. vivax*). No *ovale* malaria was detected among the residents of the camps (WHO, 2002).

From the confirmed 24 malaria cases obtained from both camps, ten microscopically positive cases were confirmed by PCR and there was a significant positive correlation between *P. falciparum* obtained with both diagnostic methods. From 276 microscopically negative cases, 10 samples were selected; 5 from each camp; which were obtained from subjects with clear symptoms of malaria. PCR results in these cases showed that 2 cases in Dar Alsalam displaced camp were positive. The results demonstrated that PCR is a sensitive and useful technology for the diagnosis of malaria in a reference laboratory, and it is very helpful in cases of low parasitemia.

In a study carried out in (2006), nested PCR was found more sensitive compared to microscopy, allowing the detection of *Plasmodium* in cases with low parasitemias, as well as mixed infections of malaria. In all instances, specimens that were PCR positive and microscopy negative were collected from symptomatic patients with a history of travel to malaria areas of endemicity (Stephanie *et al.*, 2006).

In this study, malaria occurrence was found not related to socioeconomic and ecological status or education level or use of bed nets. This does not agree with a study carried out in Zambia, where higher prevalence of malaria infection was found among the poorest population groups (Murphy and Breman 2001).

A previous study was carried in Khartoum among displaced people in

2003 showed that malaria attacks were more frequent among illiterate people than among others due to the fact that they were less aware about the disease and the preventive measures that should be undertaken (Saeed and Ahmed, 2003). In this study all the people interviewed in the two camps had good knowledge of malaria, indicating that they were familiar with the disease.

The frequency of malaria attacks during the previous years did not differ significantly between users and non-users of bednets. This may be because only a small proportion (18.8%) of the study subject owned bed nets because the majority reported that they are prohibitively expensive but now the government and organizations offer bed net almost for all the displaced people (CVHW, 2005).

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## ARABIC SUMMARY

### معدل الانتشار والتعريف الجزيئي لطفيل الملاريا في مخيمات النازحين في ولاية الخرطوم، السودان

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أجريت دراسة عرضانية وصفية في الفترة من نوفمبر 2008 الى فبراير 2009 على معسكرات النازحين بولاية الخرطوم ( معسكر دار السلام في امدرمان ، معسكر جبل اولياء في الخرطوم ) بغرض تحديد معدل انتشار الملاريا باعتبارها مناطق ضمت للنسيج الاجتماعي .

مائة وخمسون عينة دم وريدى جمعت من كل معسكر من اشخاص ظهرت عليهم اعراض الملاريا وكان توزيعها كالاتى : 59 عينة من نساء حوامل و 46 عينة لاشخاص بالغين ( 27 إناث و 19 ذكور ) ، و 45 عينة من اطفال تحت سن العاشرة ( 21 ذكور و 24 إناث ) في معسكر دار السلام بينما كانت : 48 عينة من نساء حوامل ، 52 عينة من اشخاص بالغين ( 43 إناث و 9 ذكور ) و 50 عينة من اطفال تحت سن العاشرة ( 36 إناث و 14 ذكور ) في معسكر جبل اولياء .

أختبرت جميع العينات مجهريا ثم طبقت تقنية تفاعل البلمرى التسلسلى على عشرة عينات موجبة ( 5 من كل معسكر ) وعشرة عينات سالبة ( 5 من كل معسكر ) كتأكيد لنتيجة الفحص المجهري .

مجهريا وجد ان معدل انتشار الملاريا في معسكر دار السلام حوالى ( 5 % ، 8 من اصل 150 عينة اعطت نتيجة موجبة ) و ( 11% ، 16 من اصل 150 عينة اعطت نتيجة موجبة ) في معسكر جبل اولياء . كل العشرة عينات الموجبة اعطت نتيجة موجبة لطفيل الملاريا من النوع فالسيبرم بتقنية التفاعل البلمرى التسلسلى .

كل العينات السالبة من معسكر جبل اولياء اعطت نتيجة سالبة لطفيل الملاريا من النوع فالسيبرم بتقنية التفاعل البلمرى التسلسلى .

عينتان من العينات السالبة من معسكر دار السلام اعطت نتيجة موجبة بتقنية التفاعل البلمرى التسلسلى. خلصت الدراسة الى أن الحالة الاجتماعية والاقتصادية ، والمستوى التعليمي ، واستعمال الناموسيات والحالة البيئية ليس لها ارتباط معنوى بمعدل انتشار الملاريا. كما استنتجت الدراسة أن تقنية التفاعل البلمرى التسلسلى أكثر حساسية من افلام الدم في تشخيص الملاريا و أن البلازموديوم فالسيبرم هو النوع الأكثر انتشارا في كلا المعسكرين .