



2023; 19(1):01-10

بسم الله الرحمن الرحيم
Omdurman Islamic University Journal(OIJJ)

مجلة جامعة أم درمان الإسلامية

<https://journal.oiu.edu.sd/index.php/oijj>
<https://doi.org/10.52981/oijj.v19i1.2965>



ISSN: 5361-1858

Prevalence and molecular identification of *Plasmodium falciparum* in symptomatic individuals in Nyala city, Sudan

Moussa A. Djibrine¹, Mohammed A. Boush², Abdalla A. Saeed³, Ali Mussa^{4,5}, Maki A⁵ and Khalid Hajisa³

¹ Department of Biology, Faculty of Exact and Applied Sciences, University of Ndjamen.

² Malaria, Centre for Disease Control, Malaria, Schistosomiasis and Leishmaniasis Control Program, State Ministry of Health, Nyala, Sudan.

³ Department of Zoology, Faculty of Science and Technology, Omdurman Islamic University, 14415 Omdurman, Sudan

⁴ Department of Haematology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kota Bharu 16150, Kelantan, Malaysia

⁵ Department of Biology, Faculty of Education, Omdurman Islamic University, Omdurman P.O. Box 382, Sudan

⁶ Assistant professor of epidemiology, Faculty of Medical Laboratory Science, University of Al Fashir, Sudan

To cite this article:

Moussa A. Djibrine¹, Mohammed A. Boush², Abdalla A. Saeed³, Ali Mussa^{4,5}, Maki A⁵ and Khalid Hajisa³

Prevalence and molecular identification of *Plasmodium falciparum* in symptomatic individuals in Nyala city, Sudan, Omdurman Islamic University Journal, Sudan, ISSN: 5361-1858

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Abstract

Background: Malaria is one of the most important infectious diseases in tropical and subtropical regions, and 45% of the population living in areas that suffer from stable malaria transmission in Sudan. The prevalence of symptomatic *Plasmodium* infection based on microscopy and RDTs is poorly documented in Nyala city. This study was conducted to determine the prevalence of *plasmodium* infection and molecular identification of plasmodium species in Nyala city, Sudan.

Methods: A cross-sectional study was conducted from July 2018 to December 2018 to determine the prevalence of malaria and molecular identification of plasmodium species in Nyala city, Sudan. A total of 300 suspected patients were enrolled in this study. Microscopy and RDTs were performed to estimate the prevalence and nPCR to detect the plasmodium genus and species.

Results: Of 300 patients, 113 (37.7%) were positive for malaria. *Plasmodium falciparum* was the only prevalent species detected amongst the study population. The prevalence of *plasmodium*

infection by microscopy and RDTs was 113 (37.7 %) and 106 (35.3 %), respectively. While PCR result showed prevalence of 47 (47%).

Conclusions: This study showed that the only prevalent species identified was *P. falciparum*, the findings demonstrate no existence of non-*falciparum* malaria, and children less than five years old were the most infected patients. The diagnostic performance of microscopy was better than RDTs in the diagnosis of malaria infection.

Keywords: Nyala city, South Darfur, Sudan, malaria, *Plasmodium falciparum*, nested PCR, RDTs, microscopy.

Background

Malaria remains one of the major public health threat in tropical and subtropical regions despite the intensive efforts toward malaria control interventions [1, 2]. According to the WHO report 2019, about 228 million malaria cases reported in 2018 worldwide with approximately 213 million cases (93%) of which occurred in Africa [3].

In Sudan, despite recent progress leading to an obvious drop in malaria incidence, about 75% of population is still at risk for acquiring malaria and living in areas that suffer from stable malaria transmission [4]. The prevalence of malaria has been found to vary among locations [5], possibly because of the country's variable climatic conditions and diverse topography which offer a hospitable environment for the growth and transmission of malaria parasites [6]. Until now, the majority of malaria cases in Sudan were caused by *P. falciparum* and it is responsible of most malaria deaths. In fact, about 95% of malaria cases are caused by *P. falciparum*, while the remaining 5% are caused by non-*falciparum* malaria. However, in recent years sufficient reports showed increasing rates of *vivax* malaria in different area in the country with prevalence rate up to 26.6% and 36.5% of the malaria cases [7, 8]. This data necessitates regional-specific studies in different geographical locations in Sudan to determine the prevalence of malaria and the associated risk factors. As such, the present study was conducted to determine the prevalence of *plasmodium* infection and molecular identification of *plasmodium* species in Nyala city, Sudan.

Methods

Study area

The study was carried out in in Nyala city (12° 3' 0" N, 24° 53' 0" E), the capital of South Darfur state-Sudan. Nyala cover area about 127,300-kilometer square, lying in savanna zone between latitude 9°_30,13 N and longitude 15°_27,28° E (GSCN,2012).

Study population

A total of 300 malaria suspected patients attending different Clinical Centers in Nyala city were enrolled in a cross-sectional hospital-based survey conducted during the period of July 2018 to December 2018. At the time of enrollment, demographic and clinical information was also collected.

Samples collection

Venous blood samples (2–5 mL) were collected from each participant, and dispensed into EDTA tube, mixed and stored at 4–8 °C.

Microscopy

Thick and thin blood smears were prepared on the same slide, air-dried and stained with 10% Giemsa for 10 min after the thin smears had been fixed with methanol. The smears were examined by two professional laboratory technicians. A smear was considered positive if asexual parasitaemia at any parasite density was identified.

Malaria RDT

Approximately 5 µL of collected blood was used for malaria RDT detection using SD BIOLINE Malaria Ag P.f/Pan test (Abbott, USA) according to manufacturer's instruction. This RDT is qualitative and differential test for the detection of *P. falciparum* specific HRP-II antigen and pLDH antigen specific to *Plasmodium* species

DNA extraction and Malaria PCR

DNA was extracted from 200 µL of whole blood using Geneius™ Micro gDNA Kit (Geneaid Biotech Ltd, Taiwan) following the manufacturer's instructions. The extracted DNA was kept in –20 until further use. Due to time and cost, only selected 100 samples were analysed by PCR as confirmatory test for the plasmodium genus and species. Molecular identification of *plamsoidum species* for the each selected sample was assessed by nested PCR with genus and species-specific primers designed to amplify the 18S rRNA genes as decribed previously [9].

The Nested PCR involves the first and second reaction. The first round of amplification was performed with reaction mixture of 20 µl, containing 4 µl of 5× HOT FIREPol Blend Master Mix, 1.5 µl (0.75 µM) form each primer (rPLU1 and rPLU5), 2 µl (100 ng/µl) of DNA and sterile added to make the final volume of 20 µl. The PCR programme was as follows: 95 °C for 5 min, 25 cycles of 95 °C for 1 min, 58 °C for 1 min, and 72 °C for 1 min, and a final extension period of 5 min at 72 °C. Two microliters of the first PCR product was used as DNA template for the second round of amplification using (rFAL-F and rFAL-R, rOVA-F and rOVA-R, rVIV-F and rVIV-R, rMAL-F and rMAL-R) primers. The PCR mixture and cycling conditions were exactly the same as described in the first PCR round, with only modification in annealing temperature to 62°C for 1min.

Data Analysis

The statistical analysis applied in the current study was descriptive analysis that focuses on prevalence frequency, and percentages. All statistical analysis were conducted using medcal.org/calc/diagnostic-test.php.

Results

Demographic details of studied population

Of the 300 suspected malaria patients whom recruited in this study, 188 (62.6%) were female and 112 (37.3%) were males. There were 148 (49.3%) participants younger than 20 years, 86 (28.7%) aged between 21 and 40 years, 47 15.7 between 41 and 60 years and 19 (6.3%) aged > 60 years.

Prevalence of Malaria Infection

Overall, microscopy detected slightly higher malaria infections among symptomatic patients, 37.7% (n = 113) were identified as malaria positive, while 62.3% (n =187) were parasite negative. All of the microscopy-positive samples were identified as *P. falciparum* (100%). On the other hand, the results obtained from RDTs analysis showed that 35.3% (n = 106) out of the 300 samples were positive for *P. falciparum* (Table 1).

Table 1 Microscopic, *Pf*HRP2 based-RDTs, and PCR results obtained for malaria diagnosis

Test	Total No	Positive <i>P.f</i>	Negative <i>P.f</i>
Microscopy	300	113 (37.6%)	187(%62.3)
RDT test	300	106 (35.3%)	194 (64.6%)
PCR analysis	100	47 (47%)	53 (53%)

Detection of malaria Parasite by PCR

To confirm the absence of other plasmodium species which was not detected by both microscopy and RDT, malaria parasite in randomly selected 100 samples were screened used nested PCR. The PCR results indicated that 47 (47%) samples were infected with *P. falciparum* which was the only prevalent species and none of the samples found to be positive with other plasmodium species (Figure 1 and 2).

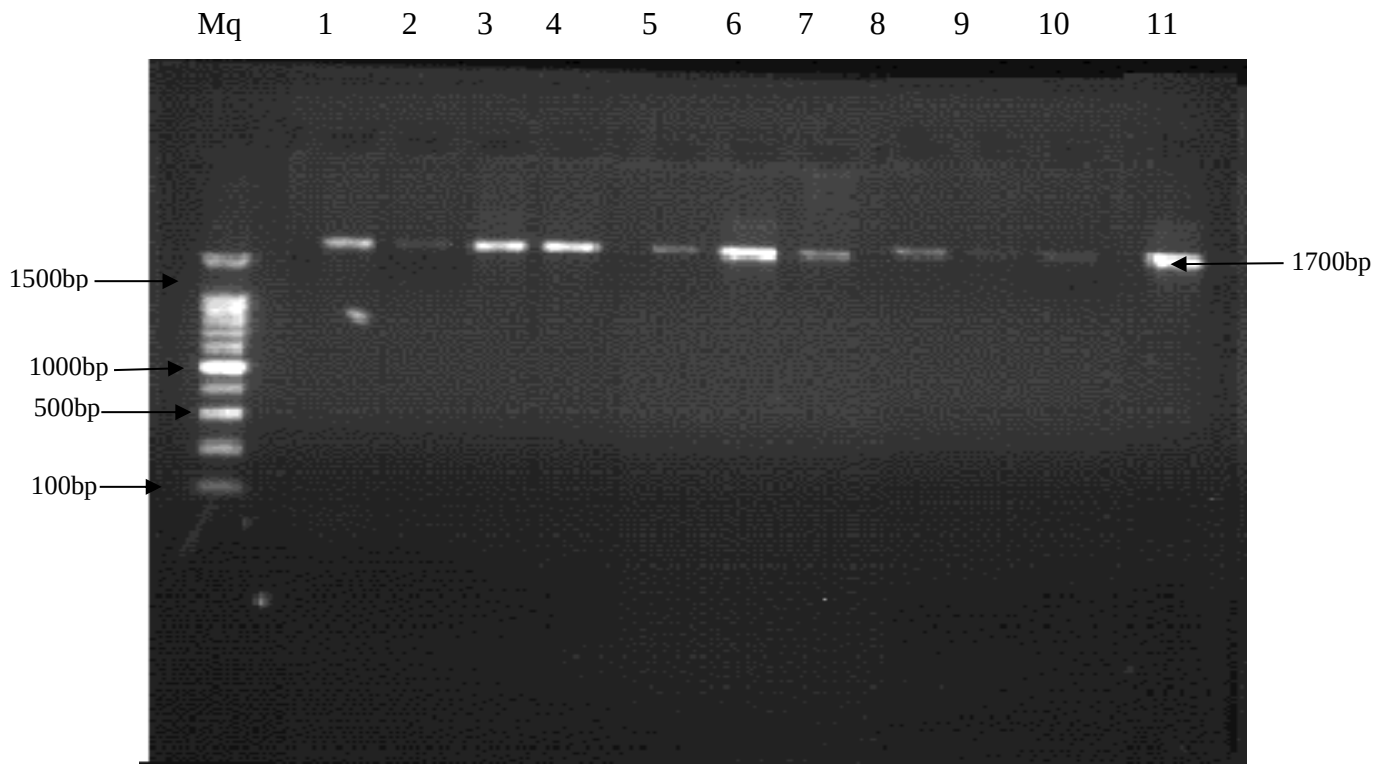


Figure 1: DNA amplification (nested 1) of *Plasmodium* genus by nPCR. Lane Mq:represents Ladder 100 bp DNA Marker.Lane 1-11 represent positive isolates plasmodium genus specific ,1700bp (rplu1,rplu5).

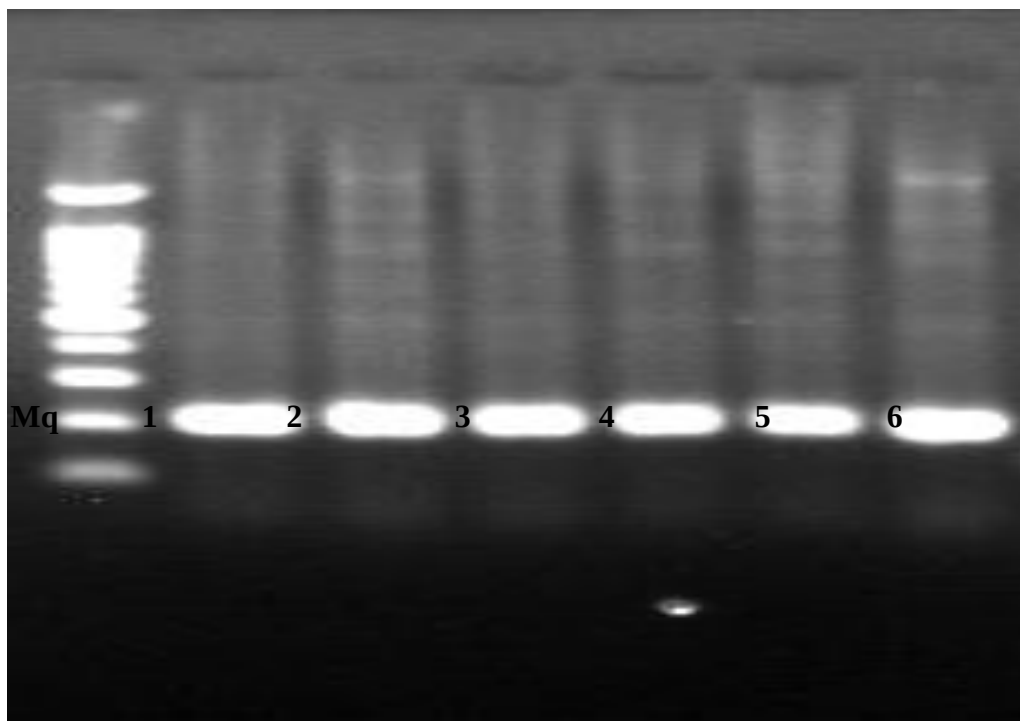




Figure 1: DNA amplification (nested 2) of *Plasmodium* species by nPCR. Lane Mq: represents Ladder 100 bp DNA Marker. Agarose 1,5% Lane 1-6: represent positive isolates samples for *P. falciparum* (206bp).

Discussion

Despite the intensive control measures, malaria is still a major health problem, with the heaviest burden and mortality is in sub-Saharan African countries [10]. In Sudan, the malaria is endemic in most areas of the country, and almost 75% of the population is at risk of acquiring the disease [4], In such country epidemiological data on malaria prevalence as well risk factors are a prerequisite for establishing any control and elimination program [11].

In this study, blood samples were collected from patients suspected to have malaria at Nyala, south Darfur, Sudan. Microscopy, RDTs, and nPCR were used as a diagnostic tool to detect the malaria parasites. The results revealed a high prevalence of the malaria infection especially *P. falciparum*, also the results were found that the most age of the patients were aged between <5-60 years, and the children whom aged between <5-20 years were higher ages infected compared to age above >60 years.

Similar findings were also reported in the Democratic Republic of Congo. The study found that the prevalence of infection was higher in females (64,8%) than in males (35,2%). And the age-specific distribution of infection in children showed that children of less than 2 years old were less infected (18.4%) compared to those aged above 2years (81.6%) [12]. In contrast, in this study the result

were in agreement with study in Ardamata displaced camp, Al-Genina-Sudan, reported by our group, we found that the prevalence of malaria infection was lower in males (27.9%) than in females (33.2%). Gender had a statistically significant association with malaria infection (COR = 1.55, $p = .042$), indicating that the males were 55% more likely to have malaria infection than the females [13]. But the present study, were indicated that the prevalence of malaria infection was high in females (62.7%) than males (37.3%).

The present study was also contradicted with study reported by Zakya *et al.* [14]. Who showed that the ratio of the gender in a total of 341 samples 196 (57.7%) were male and 100 (29.3%) were female, and their mean age was 29, 3 years (SD.19, 7), of these patients. The results also indicated that 113 (37, 7%) positive malaria infection by microscopy, and 187 (62.3%) were negative for malaria infection, and *P. falciparum* was the most prevalent species. Our research group on the other hand reported similar finding in Ardamata displaced camp, Al-Genina-Sudan, we found that out of 380 samples , 232 (61.1%) were positive for malaria, and the most prevalent species was *P. falciparum* amongst the study population [13]. However, our results was disagreement with the study reported by In Democratic Republic of Congo [12], who found a relatively high prevalence rate of *P. falciparum* was the most prevalent 141out of 124 (99.2%).

Assessment of malaria burden by microscopy and RDT demonstrated that the overall prevalence of *P. falciparum* infection among suspected malaria patients was 37.67 % and 35.33 % respectively. The microscopic prevalence in this study was higher than the 23.5% reported in 2019 from the same city [8], and the 22% among adult symptomatic patients presented to Kassala Hospital, eastern Sudan [15]. However, the prevalence was far lower than the 68.5 % and 72.50% reported among suspected malaria cases in Aljabalain hospital and Aljabalain military hospital, central Sudan [7]. The variation in the prevalence of the diseased was also noticed when sensitive diagnostic method (PCR) was applied. Even though a remarkably high prevalence 61.1% was obtained when PCR assay was applied [16]. In contrast, in study conducted by our group in Khartoum state, in 2018, PCR result showed a prevalence of 44.1% less than the current study [9]. In the present study, microscopy resulted high sensitivity than RDTs, from 300 samples 113(37%) were positive, and were 187 (62.3%) were negative infection of malaria parasites. while the RDTs from 300 samples 106 (35,3%) were positive, and 194 (64.6%), were negative infection for malaria parasite. The present study disagreement with previous report [14]. Who found that, microscopy resulted in lower sensitivity (74.3%) than the RDT (80.7%) for the diagnosis of malaria.

The result of molecular diagnosis (PCR) showed that a high prevalence of *P. falciparum*, from 100 samples analyzed by PCR 47(47%) were positive for *P. falciparum* infection, and 53(53%) were

negative samples. While in Cameroon, the prevalence of *P. falciparum* was 31%, 45%, and 54% by microscopy, RDT, and PCR, respectively [1]. Also, another study in India reported that, the prevalence of *P. falciparum* was 52% by microscopy, and 45% by RDT, while by PCR was 42% [6]. However, in Tanzania, the study reported prevalence of 73.3% by PCR, 40.8% by RDT, and 36.3% by microscopy [17]. This variation between different areas in the same country or even within the same city could be due to the study period, which in the peak season is expected to report high prevalence.

Conclusion

P. falciparum carriage is still common among Sudanese, notably in Nyala City in western Sudan, despite modern control programs. Therefore, more work is required, particularly in remote rural regions. Despite the low frequency with which this kind of malaria transmission occurs, it is nonetheless important to continue screening blood donors. It is impossible to eradicate vectors in forested environments. However, the transmission rate may be lowered by using insecticide-treated bed nets and treating people with asymptomatic infections or clinical malaria.

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