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COLORIMETRIC DETECTION OF PLASMODIUM VIVAX IN PATIENT'S URINE USING MSP10 OLIGONUCLEOTIDES AND GOLD NANOPARTICLES

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ABSTRACT

Background: In Sudan and elsewhere, diagnosis of malaria relies entirely on examination of blood taken from suspected individuals. In this study an attempt to examine urine for detection of *P. vivax* infection is carried out. **Methodology:** A total of 35 urine samples from *P. vivax* positive patients and 25 negative individuals were heated by thermocycler and cooled were mixed with *P. vivax* merozoites surface protein 10(MSP10) and AuNPs. **Results:** The results showed that the urine of all *P. vivax* positive patients turned red color due to stabilization of the gold nanoparticles. **Conclusion:** this indicate that MSP10 and nanoparticles are sensitive enough to detect *P. vivax* and can consequently be recommended as diagnosis techniques.

KEYWORDS: *Plasmodium vivax*, Gold nanoparticles (AuNPs), Merozoite surface protein 10 (MSP10).

INTRODUCTION

Malaria infection caused by *Plasmodia* species that belong to phylum Apicomplexa. In the world 2 billion at risk infection, 1.5-3.5% people particularly children. They are transmitted through the bite of an infected female Anopheles' mosquito.[1-3] Five species of Plasmodium can infect humans[4], most deaths are caused by *P. falciparum*, because *P. vivax*, *P. ovale* and *P. malariae* generally cause milder form of malaria. The species *P. knowlesi* rarely causes disease in human.[5]

Malaria in Sudan is a major public health problem. The country is hit by an estimated 50% of all malaria cases in the World Health Organization's Eastern Mediterranean Region survey with an estimated 7.5 million cases resulting in 35,000 deaths per year.[6-8] The disease accounts for 20% of all hospital death. The malaria case fatality rate for pediatric hospitals ranges between 5% and 15%. According to the Malaria Indicator Survey (MIS), in October 2005 the prevalence of malaria among children under the age of 5 years, ranged between 0.4-15.5% and between 3.7%-10.3% for pregnant women.[9,10]

Malaria is diagnosed by the microscopic examination of blood using blood film, this method is considered gold standard, or with screening rapid diagnostic tests and polymerase chain reaction to detect the parasites DNA have developed but are not widely used in area where malaria is common due to their cost and complexity.[11-13]

In this pilot study we use merozoite surface protein 10 (MSP10) gene and gold nanoparticles can play inexpensive, rapid and less time consume.[14]

The objective of this study was to evaluate this method for detection of DNA in urine using gene MSP10 primers and AuNPs in diagnosis of *Plasmodium vivax* in comparisons to gold standard method.

MATERIALS AND METHODS

A total of 35 *P. vivax* –infected patients (20 from Blue Nile state,10 in Kassala state and 5 in Khartoum state) and 25 negative individuals were screened (Table 1). Blood samples were taken, stained with Giemsa and examined under microscope (100x) for detection of *P. vivax*

Once urine was collected at room temperature, dipsticks were carried out to determine urine PH and the presence of protein. Each urine sample was centrifuged at 15.000 rpm for 5 minutes to remove sediments. The urine samples were diluted 1:16 with PBS diluted samples. 50 µl of each diluted urine sample was heated at 95 °C for 30 seconds, using thermocycler. Samples were cooled at room temperature for ten minutes, 10µl of MSP10 oligonucleotides and 20µl of 0.25 M NaCl were added. The sample was heated at 59°C for two minutes and allowed to cool at room temperature for ten minutes. Finally, 50µl citrate of reduced AuNPs were added two minutes later, the system was read visually.

Chemical and reagent

Citrate reduced gold 15 nm nanoparticles prepared in lab (citrate synthesis of gold nanoparticles)

Na citrate HAuCl 4 and KCL PBS and NaCl were purchased from (sigma –Aldrich).

MSP10 oligonucleotides

The two MSP10 oligonucleotides utilize in this study. The oligonucleotides are a sequence N. Terminal: 5´AGCCATGGAACGTGCTAAGTGCAACA3´ and C Terminal 5´CACCATGGAACAGTTT ATCCTGAAGAC3' were purchased from macrogene company.

Table 1: Urine samples were collected from 3 states in Sudan.

Urine samples	state	quantity
Positive for *P.vivax*	Kassala State	0
Positive for *P. vivax*	Blue Nile State	20
Positive for *P.vivax*	Khartoum State	5
Negative control	Khartoum State	25

RESULTS

In figure (1) showed positive result for *P. vivax*. That mean all the samples stabilized the gold nanoparticles and maintained red color while in figure (2) showed negative controls induced aggregation and allow color change to blue, this color can be distinguished by naked eye (colorimetery).

Color change and detection of *P. vivax (red in +ve control & blue in –ve control)*

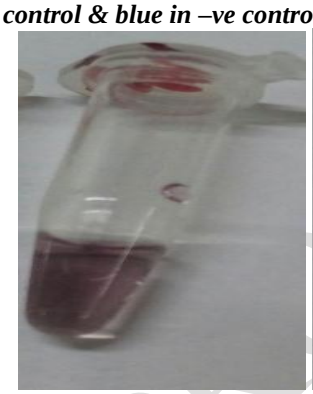

Figure (1): Positive for P. vivax.

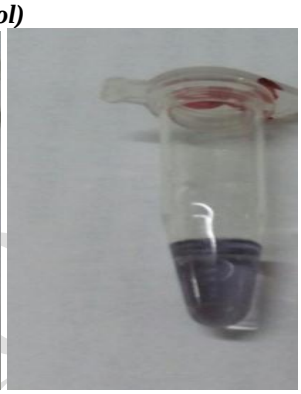

Figure (2): Negative control

Table 2: Accuracy of AuNPs in comparison with Blood film in detecting *P. vivax.*

		Blood film (GSTD)		
		Positive	**Negative**	**Total**
AuNPs	**Positive**	35	0	35
	Negative	0	25	25
	Total	35	25	60

DISCUSSION

The main finding of the current study was that the sensitivity of MSP10 oligonucleotides in detecting DNA in urine samples depend on quality of urine.[14] DNA in fresh urine is good rather than stored urine.[15] So urine contains smaller double strand DNA of 150-250 nucleotide size that can interact with nanoparticles and still be utilized as valid source of microorganism´s DNA.A similar study was conducted in the United States of America and the results were the same as ours.[14]

Merozoite surface protein 10 (MSP10) is one the asexual stage proteins of *P. vivax* linked to erythrocyte invasion.[14,16] We used two primers in our study N terminal and C terminal.

Currently most commercially rapid diagnostic tests (RDTs) such as histidine – rich protein two (HRP2), aldolase and *P. vivaxs'* lactate dehydrogenase (pLDH) employ monoclonal antibodies in their identification that is reported variable sensitivity and specificity.[13,17,18]

This test can be detecting a positive urine sample in patient with low parasitaemia. Whereas lack of correlation between blood and urine DNA, this test has many other advantages; simplification of the process, rapid, does not require extensive lab skills and low cost.

CONCLUSION

According to previously, presented of this study it is clear that is first RDT utilizing urine samples rather than blood and employing nanoparticles. Using AuNPs and MSP10 oligonucleotides to detect *P. vivax* in urine.in addition to present a safe, rapid and cheap tool to diagnosis one of most common form of malaria.

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