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RESEARCH ARTICLE

New method of DNA extracted from thick film blood sample slides

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Abstract:

Objective: To evaluate the modified rapid DNA extraction from blood films, and the development and application of reliable methods to detect parasites.

Methods: DNA was extracted from thick film blood sample slides, sample was took by razor in a eppendorf tube. 1ml ethanol 100% was added for 1minute and was then let to dry for 10 minutes. 80 µl methanol 100% was added and was let for 15 minutes then centrifuged at 12000 rpm for 3 minute. The precipitated part, was discharged and was let to dry for 15 minutes. 50 µl Distilled water was added boiled for 15 minutes with shaking every 3 minutes then centrifuged at 12000 rpm for 5 minutes and take the supernant to clean tube.

Results: A total of 30 cases were studied, 17 males and 13 females with age ranges between 4 to 57 years. The all sample use give Positive result when use new method of extraction. comparison this method with other stander method give the same result but this method rapid DNA extraction from blood films This is the first study to be done in Sudan to diagnose malaria from slide by PCR. More simple and cheep techniques.

Key Words: Malaria, PCR, Giemsa stain

Introduction

Molecular tests

Efficient molecular tools are now increasingly used for vector vector identification and transmission studies as endemic countries scale up intervention against malaria. Simple and inexpensive methods are needed to help establish application of such powerful tools in resource poor countries, which are the stronghold for malaria.

During the past few years the polymerase chain reaction (PCR) has become a major research and diagnostic technique in medicine. An attractive future of PCR is that, unlike other molecular techniques, high quality DNA is not required for successful analyses of clinical specimens. Since minute quantities of degraded DNA can serve as the template for the reaction, the method is ideally suited to a template extracted from archival clinical specimens (Poljak et al., 1995).

Detection of parasite genetic material through polymerase-chain reaction (PCR) techniques is becoming a more frequently used tool in the diagnosis of malaria, (Beck *et al.*, 1997). The extraction of DNA from such samples is a critical step and several methods are being used. In malaria field studies involving detection of parasite DNA and genotyping of the infecting parasites, mostly finger prick blood samples are collected (Felger *et al.*, 1994). DNA is routinely isolated from these samples either by phenol:chloroform extraction or by a rapid boiling method (Foley *et al.*, 1992). Methods for DNA preparations from blood samples in studies of epidemiological scale have to fulfill the following criteria: rapid preparation, high reliability, production of DNA of good quality for long-term storage, avoidance of cross-contamination and reasonable costs.

Method of DNA Extraction

Methanol Method from Filter Paper

DNA was extracted from filter paper blood samples by cutting an approximately 4 mm^2 of the sample which was placed in 100 µl methanol, air dried, and incubated at 95 to100 ºC in 50 µl of water for

15 minutes. During incubation, the tube was subjected to high-speed vortex three times. Five µl of resulting solution was used as template for PCR.

Method of Sodium Phosphate Dehydrates

To each 20 µl of infected blood, 500 µl of ice cold $Na_2HPO_4(2)H_2O$ (pH 8), were added, vortexes, and centrifuged (eppendorf micro centrifuge 5410) for 10 min at 14 000 rpm. Then the supernatant was discarded. This step was repeated 2-3 times. Finally, 50 µl of sterile double distilled water was added to the pellet which was vortexes, boiled for 10 minute then centrifuged for 10 minute at 13000 (rpm), the supernatant which contained the DNA was used immediately.

Molecular tests

DNA extraction modified method

DNA was extracted from thick film blood sample slides, sample was took by razor in a eppendorf tube. 1ml ethanol 100% was added for 1minute and was then let to dry for 10 minutes. 80 µl methanol 100% was added and was let for 15 minutes then centrifuged at 12000 rpm for 3 minute. The precipitated part, was discharged and was let to dry for 15 minutes. 50 µl Distilled water was added boiled for 15 minutes with shaking every 3 minutes then centrifuged at 12000 rpm for 5 minutes and take the supernatant to clean tube.

Premix for the PCR reaction outer gene

Reagents for one mix

PCR was performed in 30 µl volumes which contain s 1X PCR , 3 µl 10X Buffer [10 mM Tris-HCl, pH 9, 0.1% Triton X-100, 50 mM KCl, 0.2 mg/ml BSA], 1.8 µl (25 mM $MgCL_2$), ($MgCl_2$, Applied Bio system), 3 µl (10 mM each of the dNTP (Gene Amp® dNTPs, Applied Bio system), 2 µl 10X of each sense PS500-A and antisense primers PS500-B, 1.5 U of AmpliTag Gold (Applied Bio system), 5 µl of the Template DNA; and the reaction volume was completed to 30 µl by adding 11.2 µl of ddH_2O (Kublin, 2002).

Amplification of outer gene

PCR for the detection of Dihydropteroate synthtase (DHPS) gene in field isolates for all reactions was done using Model Gene Amp® France PCR system (Applied Bio system) in the following conditions: one cycle at 94 ºC for 10 minutes; 40 cycles at 94 ºC for 1 minute, 50 ºC for 2 minute, and 72º C for 2 minute; and a final extension at 72º C for10 minutes. PCR was carried out using outer primers PS500-A and PS500-B (Duraisingh *et al.*, 2000).

Premix for the PCR reaction of Inner gene

Reagents for one mix

PCR was performed in 30 µl volumes which contains 1X PCR , 3 µl 10X Buffer [10 mM Tris-HCl, pH 9, , 0.1% Triton X-100, 50 mM KCl, 0.2 mg/ml BSA], 1.8 µl (25 mM $MgCL_2$), ($MgCl_2$, Applied Bio system), 3 µl (10 mM each of the dNTP (Gene Amp® dNTPs, Applied Bio system), 2 µl 10X of each sense PS500-D1 and antisense primers PS500-D2, 1.5 U of AmpliTag Gold (Applied Bio system), 2 µl of the outer PCR product; and the reaction volume was completed to 30 µl by adding 14.2 µl of ddH_2O (Kublin, 2002).

Amplification of inner gene

PCR for the detection of DHPS gene in field isolates for all reactions was performed using Gene Amp® France PCR system (Applied Bio system) in the following conditions: one cycle at 94 ºC for 10 minutes; 35 cycles at 94 ºC for 1 minute, 45 ºC for 1 minute, and 72º C for 2 minute; and a final extension at 72º C for10 minutes. Amplification out using outer primers PS500-D1 and. PS500-D2 (Duraisingh *et al.*, 2000).

Primer sequences used to amplify dihydropteroate synthtase (DHPS) gene

Primer used	Primer Sequence (5' to 3')	Position
PS500-A	5'-GGGCCCAAACAAATTCTATAGTG-3'	DHPS Outer
PS500-B	5'-GGCCGGTGGATACTCATCATATA-3	DHPS Outer
PS500-Dl	5'-GCGCGCGTTCTAATGCATAAAAGAGG-3'	DHPS Inner
PS500-D2	5'-CCCGGGTAAGAGTTTAATAGATTGATCAGCTTTC TTC-3'	DHPS Inner

Results and Discussion

A total of 30 cases were studied, 17 males and 13 females with age ranges between 4 to 57 years. The all sample use give Positive result when use new method of extraction. comparison this method with other stander method give the same result but this method rapid DNA extraction from blood films This is the first study to be done in Sudan to diagnose malaria from slide by PCR. More simple and cheep techniques.

In this study, PCR has shown 100% sensitivity and accuracy in examination of blood film for malaria. The microscopic examination was done by using slides stained with Giemsa stain. This method is easy and not expensive and can be used to train students in the laboratory. In case of low parasitemia or negative slide performed by microscopic method, the samples were rechecked from these slides to be tested in the PCR which gave a positive results because of its more sensitivity100% than the microscopic method 87%. This primers (outer and Inner) can not detect the different stages of parasite.

PCR was more sensitive than microscopic and ICT, however the sensitivity of PCR is one parasite in 1 µl of blood, but it did not count the parasites number. PCR is useful technique of malaria diagnosis in case of low parasitemia where microscopic examination fails to diagnose the case.

In addition Processing time for the new method was shorter than the other method. There is no significant difference in DNA template from the new method and the other extraction protocol. Therefore the new method can be recommended as an alternative in settings with limited resources.

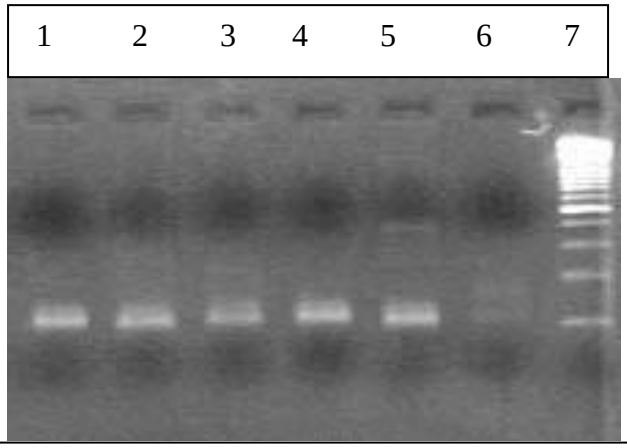

Fig. 1: Detection of *P. falciparum* using nested PCR method.
Lane 1: positive control. Lanes 2 -5 Positive amplification (250 bp) PCR products. Lane 6. PCR product (negative control) and Lane 7 DNA marker.

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