Short Communication

Detection of malaria parasitaemia by polymerase chain reaction with an internal control

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Abstract

Polymerase chain reaction was carried out on the 18S rRNA gene of *Plasmodium. falciparum* to detect the presence of falciparum parasitaemia using human γ-interferon as an internal control. Twenty two out of 26 microscopically positive specimens were positive by PCR. Of the 25 microscopically negative specimens, 21 were negative by PCR while in 4 specimens the internal control was also negative.

Key words: *Plasmodium falciparum*, PCR diagnosis, malaria.

Current methods of polymerase chain reaction (PCR) detection of malaria parasitaemia are based on the detection of the specific amplified fragment. However, PCR may fail due to many reasons, for example, the presence of inhibitors like haem. This phenomenon is more important in PCR for detection of blood-borne parasites, where contamination with haem can occur. The use of external controls cannot reliably confirm that absence of PCR products in the individual tubes were not due to inhibition of PCR in them. Current PCR assays for the diagnosis of falciparum parasitaemia are unable to distinguish PCR failures from true negative specimens. The purpose of the study is to attempt to test the use of PCR with an internal control for the detection of *Plasmodium falciparum*. This was carried out by multiplexing the amplification of the 18S ribosomal DNA of *P. falciparum* with the amplification of the human γ interferon gene as the internal control.

Twenty six pretreatment whole blood specimens confirmed positive for *P. falciparum* by light microscopy (parasite count ranging from < 40 - 132,320 asexual stages/0 - 560 sexual stages per µl) were used. As controls, 25 blood specimens confirmed as negative by examination of 200 fields at 1,000x magnification, of thick blood films were used. The blood samples were lysed with 0.15% saponin/phosphate buffered saline at 37°C for 20 minutes, washed with PBS once, digested with Proteinase K for 2 hours, heated at 100°C for 10 minutes, vacuum dried and resuspended in sterile distilled water. Primers specific for *Plasmodium falciparum*, rFAL1 (TTAAACTGGTTTGGGAAAACCAAATAT-ATT) and rFAL2 (ACACAATGAACTCAATCAT-GACTACCCGTC) (Snounou *et al.*, 1993) would resolve a 205 bp fragment. For the internal control, primers Ifn150F (TCTTTTCTTTCCCGATAGGT) and Ifn150R (CTGGGATGCTCTTCGACCTC) (Frye *et al.*, 1989) which amplifies a 150 bp size band from the human γ-interferon gene, were used. PCR was carried out using 50 pmol of each primer, 3 mM $MgCl_2$, 10 mM Tris-HCl (pH9.0), 50 mM KCl, 0.1% Triton X-100, 2.5 U Taq polymerase and DNA from an equivalent of 10 µl blood in a 50 µl reaction volume. PCR was carried out in a GeneAmp 9600 PCR System Thermal cycler (Perkin-Elmer Cetus, USA) using a manual hot start at 99.9° C for 3 mins and 85°C till Taq polymerase was added. Denaturation was carried out at 94°C for 10s, annealing at 55°C for 10s and extension at 72°C for 10s for 40 cycles with a final extension step at 72°C for 7 mins. 10 µl PCR products were resolved on a 2% agarose gel with ethidium bromide staining and visualised using a uv transilluminator (Figure 1).

Out of the 26 microscopically positive specimens, 22 (84.6%) were positive by PCR. Twenty one microscopically negative specimens were negative for

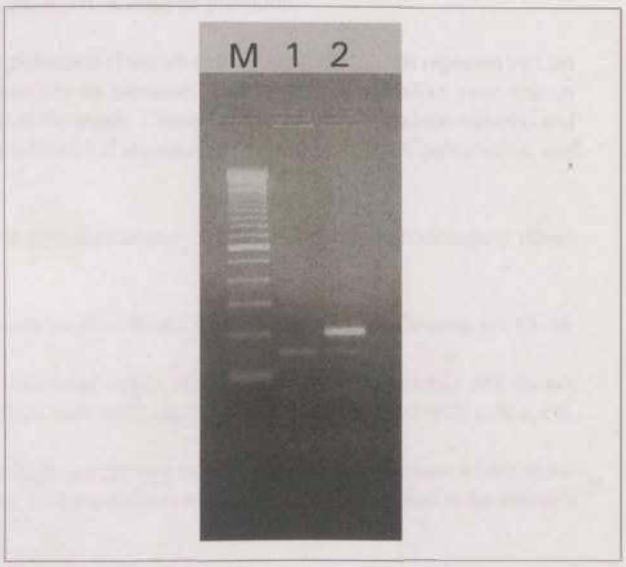

Fig. 1. Detection of *Plasmodium falciparum* malaria using PCR amplification of 18S ribosomal DNA, with human γ interferon gene as internal control. M = 100 bp ladder; Lane 1 = Negative specimen showing 150 bp band of internal control; Lane 2 = Falciparum malaria positive specimen showing both the 205 bp of the 18S rRNA gene of *P. falciparum* and 150 bp band of internal control.

falciparum parasitaemia by PCR while 4 specimens did not give the internal control band (150 bp) suggesting PCR failure.

The study was limited by the small sample size and that the bands on the gel were read visually. However, the lack of sensitivity of the assay needs to be addressed further. There did not appear to be a relationship between low parasitaemia and the inability to detect *P. falciparum* by PCR. The assay was able to detect falciparum parasitaemia in a specimen with 100 parasites per μl. The possible reasons for the low sensitivity could be due to multiplexing, the need for further optimization of the assay or random errors in the study. However, the assay was not as sensitive as our nested PCR assay (Khoo *et al.* ,1996). Foley *et* al. (1992) showed that the sensitivity of direct PCR followed by detection of PCR products by agarose gel electrophoresis could detect 500 parasites per μl but not 200 parasites per μl although the latter was detectable by nested PCR. There were no specimens found positive by PCR but negative by microscopy. PCR based diagnosis has the advantage of being less subjective. Our method was able to alert us of PCR failure as a cause of negativity by PCR in our specimens. Investigations of the cause of the PCR failures in the 4 specimens could not be done as there was no more template available for further testing. More studies on this would be useful. The method is unable to provide information on the viability of the parasites. Further work is needed to improve the sensitivity of the assay (which could include the use of more sensitive methods for detection of PCR products), to incorporate the detection of other species of malaria parasites and to automate the process.

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