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USEFULNESS OF PCR IN SURVEILLANCE OF MALARIA INFECTION IN MELGHAT REGION (M.S.) INDIA

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ABSTRACT

The present study was an attempt to diagnose the usefulness of PCR in surveillance of malaria infection in Melghat (M.S.) India. The 23 complicated cases where the morphologic characteristics of malaria parasites fail were selected for the present study. The diagnostic band for *P. vivax* infection was represented with size of 120 bp while for *P. falciparum* with size of 205 bp. From study, PCR was observed to be more sensitive and specific than all other techniques for confirmation of malaria in complicated cases where morphological characteristics of malaria parasites fails due to blood film parasitaemia below the detectable level.

KEYWORDS :*PCR, Malaria and Melghat Region.*

INTRODUCTION :

Melghat is a mostly remote area of Amravati district of Maharashtra (India) located between 20^0 51'- 21^0 46' N and 76^0 38'- 77^0 33' E in Northern part. Due to heavy rain and existence of conditions which help breeding of mosquitoes, area is badly infested with malaria. According t WHO (1986), Malaria continues to remain a major public health problem in many countries even after more than four and half decades of organized anti-malaria control measures, initiated after the declaration of World Health Assembly in 1955 (Kondrashin and Rashid, 1987). Molecular methods have shown a promise in this aspect. Analysis of DNA by the polymerase chain reaction (PCR) can be a useful tool for diagnosis of malaria when the results of conventional techniques are negative, especially since PCR allows accurate species identification and can detect low level parasitaemia. PCR, a more sophisticated technique, requires infrastructural support, is expensive and time taking than the conventional thin smear examination and immuno-chromatography (Morassin *et al.*, 2010).

The present study was attempted to diagnose the usefulness of PCR in surveillance of malaria infection in Melghat (M.S.) India.

METHODOLOGY

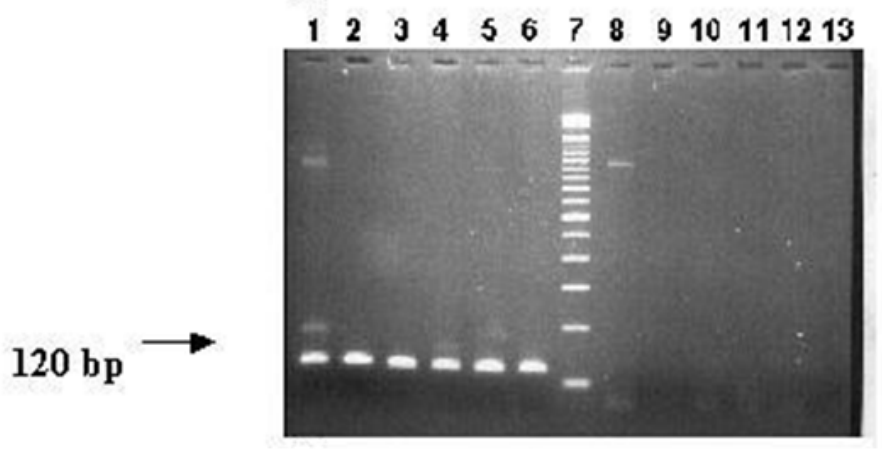
The present study was conducted during February 2012-January 2014. The 23 complicated

malarial cases were selected for confirmatory molecular diagnostic tests. The molecular tests such as PCR can detect parasites in specimens where the parasitaemia is below the detectable level of blood film examination. The Agarose gel (2%) analysis of a PCR diagnostic test for species-specific detection of *Plasmodium* DNA was performed using nested primers of Snounou et al., (1993). Plasmodium genomic DNA is extracted from 200 µl whole blood using the QIAamp Blood Kit. Detection and identification of Plasmodium to the species level is done with a two step nested PCR using the primers of Snounou *et al.*, (1993). In the first step (PCR 1), 1 µl of extracted DNA is amplified using genus specific primers; in the second step (PCR 2), 1 µl of PCR1 amplification product is further amplified using species specific primers. Ten µl of each PCR2 amplified DNA product is separated by 2% agarose gel electrophoresis stained for 15 min with Ethidium bromide and visualized by UV illumination (Zakeri *et al.*, 2002).

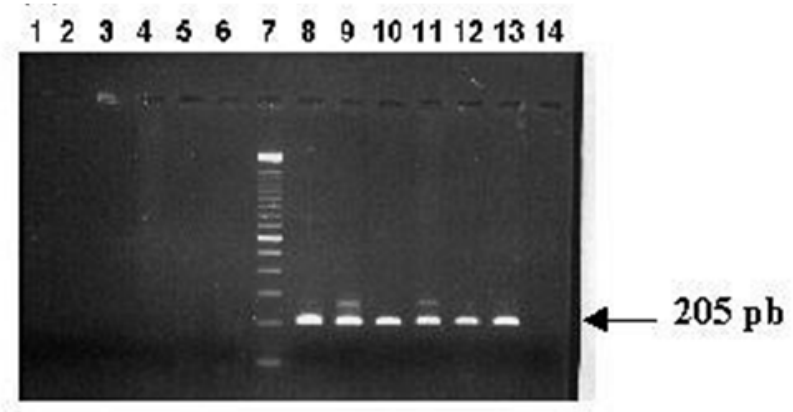
RESULTS AND DISCUSSION

The diagnostic band for P. vivax was represented with size of 120 bp while for *P. falciparum* with size of 205 bp (Figure.1 and 2). The findings were in well agreements with Snounou *et al*. (1993), Machado et al., (1998) and (Zakeri et al., 2010). It is well accepted that molecular methods are developed and became more applicable in routine diagnosis. These methods also provide the information about drug-resistance and genetic diversity of malaria parasites that led scientists to apply these methods more commonly. Snounou et al. (1993) have reported that PCR has proven to be more sensitive and accurate than routine diagnostic microscopy in detection and identification of the parasites. WHO (2000) reported that PCR is more sensitive and specific than all other techniques. It is, however, a lengthy procedure that requires specialized and costly equipment and reagents, as well as laboratory conditions that are often not available in the field. Humar *et al.* (1997) have reported that it is difficult to evaluate the specificity of PCR for the identification of *P. falciparum* or *P. vivax* in a single sample with very low Plasmodium concentration; however, the evaluation of PCR method together with the thin smears can be accepted as the gold standard (Tantarpale and Tantarpale 2014).

Figure.1: Schematic representation of agarose gel electrophoresis of nested PCR products is from clinical specimens using species-specific Oligonucleotide pairs for A (*P. vivax*), B (*P. falciparum*)



(A) The representative microscopically *P. vivax* diagnosed samples, which were positive by using *P. vivax*-specific primers (left panel), and negative by *P. falciparum*-specific primers (right panel).



(B) The representative microscopically *P. falciparum* diagnosed samples, which were positive by using *P. falciparum*-specific primers (right panel), and negative by *P. vivax*-specific primers (left panel)

CONCLUSION

In concluding the above results, it is cleared that the PCR is observed to be more sensitive and specific than all other techniques for confirmation of malaria in complicated cases where Morphologic characteristics of malaria parasites fails due to blood film parasitaemia was below the detectable level . It is, however, a lengthy procedure that requires specialized and costly equipment and reagents, as well as laboratory conditions that are often not available in the field.

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