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Use of Molecular Diagnostic Techniques to Determinate the Epidemiology of Malaria Parasites in North Eastern Nigeria

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ABSTRACT

The emergence of resistance to all antimalarial drugs in clinical use is now making it necessary to discover the markers responsible for the resistance. The principal aim of this research is the use of molecular diagnostic techniques to Determine the epidemiology of malaria parasites. Thirty blood samples were analyzed by microscopy and molecular techniques to monitor the relative efficiency in malaria diagnosis. Molecular analysis revealed 28 out of 30 samples as positive for malaria while Microscopic analysis revealed 27out of 30 samples as positive malaria parasite. The molecular analysis was particularly useful to unveil parasites presence in infections not detectable by blood smear analysis.

Keywords: Molecular Diagnostic Techniques, Epidemiology, Malaria Parasites

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INTRODUCTION

Malaria is a dangerous infectious disease, caused by any one of the four (4) species of protozoan parasites of the genus Plasmodium, known to infect humans. Four main species of *Plasmodium* cause disease in humans: *Plasmodium falciparum*, *P. vivax*, *P. ovale* and *P. malariae.* In addition, a simian parasite, *P. knowlesi*, was recently reported to occasionally infect humans (Sabbatani *et al*., 2010)[1]. *P. falciparum* is the most virulent and leads to most of the deaths while, *P. vivax* is the most widespread (Flannery *et al*., 2013)[2]. The complicated life cycle of these parasites alternate between a vertebrate host (human) and an invertebrate vector (*Anopheles gambiae*).

While the World Health Organization (W.H.O) reveal that people living in the poorest countries of the world are the most vulnerable to malaria (W.H.O, 2014)[3], records show an estimated 3.4 billion individuals, almost half of the world's population – at risk of getting infected with the disease (Tekwani & Walker, 2005; W.H.O, 2014)[4]. In 2012, an estimated 207 million cases and roughly 627,000-deaths due to malaria were recorded (Noubiap, 2014)[5]. However, there are indications that malaria mortality had reduced by 42%, due to an increase in prevention and control measures globally, and by 49%, in the WHO African region since 2000 (W.H.O, 2014). Despite these reductions, 90% of all malaria mortality occurred typically amongst children below the age of five in the WHO African Region, (W.H.O 2014). It is, therefore, thought that malaria imposes a heavy social burden that has delayed economic development in regions where it is endemic.

In order to address sensitive research questions such as the distribution and sensitivity/resistance to drugs or virulence of malaria parasite in the study area, it is important to consider the diagnostic techniques used. Conventional diagnosis such as smear and/or rapid diagnostic tests (RDTs) are the only methods used so far. It is also important to realize that the 'correct identity' of protozoan parasites such as Plasmodium/Malaria parasite is quite challenging because morphological criteria are often not used as identification tools (Hyde, 2011)[6]. However, accurate identification of the parasite is vital to the understanding of the epidemiology of the disease, and particularly, treatment procedures. Molecular epidemiology of parasites involves the application of DNA based techniques as tools to understand the epidemiology and diversity of the parasite in question (Hyde, 2011). Amplification techniques that allow the detection of specific parasite species or genotypes have proven useful for the purposes of clinical diagnosis and for assessment of parasite distribution and prevalence (Monis et al., 2002)[7].

MATERIALS AND METHODS

Study Area

This study was conducted in Damaturu Local Government Area of Yobe State. It has area of 2,366km^2 and a population of 88, 014 at the 2006 census.

Ethical clearance: Ethical clearance was sought from Yobe State Ministry of Health.

Sample collection: Blood sample was collected from patients visiting medical laboratory in specialist hospital in the study area, and sample were preserved in EDTA containers and/FTA Carts before culture.

Sample size: 30

Sample size calculation: The target population from which the samples were randomly selected was 88, 014. We assumed the margin error of 18% and confidence level of 95%.

Blood examination: The Giemsa-stained thick blood smear technique was used for malaria diagnosis in all of the samples in the present study.

Parasite culture: Parasites were cultured and maintained in complete Roswell Park Memorial Institute (RPMI 1640) (Gibco) media as described by (Matthews *et al.,* 2013)[8]. 12.5 cm^3 and 25 cm^3 culture flasks was used and final culture volumes of 10 ml and 5% final haematocrit and 20 ml and 10% final haematocrit was maintained.

Parasite Isolation from blood samples:

Malaria parasites were isolated from the blood samples of infected individuals in the study area by saponin lysis.

DNA extraction

The parasite was lysed by chemical means (saponoin) and PCR inhibitors such as haem in blood and heparin was removed.

Primer Designed: Sequence of nucleotide was identified, isolated and tagged with labels. Oligonucleotides was used and primers that were complimentary to specific regions of DNA.

RFLP-PCR (Strain typing): Restriction enzymes EcoR I was used to cut restriction sites identified in the target DNA, followed by enzymatic amplification of DNA through successive cycles of denaturation, annealing and extension steps. The PCR product was detected by agarose gel electrophoresis with ethidium bromide before visualizing under UV illumination.

RESULTS

The table 1 below showed the results of thirty samples collected and were diagnosed using two different diagnostic methods of determination of malaria parasite.

Table 1: Detection of parasite from blood samples

S.N.	Microscopically Diagnosis	Molecular Diagnosis
1	*P. falciparum*	*P. falciparum/P. vivax*
2	*P. falciparum*	*P. falciparum*
3	*P. falciparum*	*P. falciparum*
4	*P. falciparum*	*P. falciparum*
5	*P. vivax*	*P. falciparum/P. vivax*
6	*P. ovale/P. malariae*	*P. malariae*
7	*P. falciparum*	*P. falciparum*
8	*P. vivax*	*P. vivax*
9	*P. falciparum*	*P. falciparum*
10	*P. falciparum/P. malariae*	*P. falciparum*
11	*P. falciparum*	*P. falciparum*
12	*P. vivax*	*P. vivax*
13	*P. falciparum*	*P. falciparum*
14	*P. falciparum*	*P. falciparum*
15	*P. falciparum*	*P. falciparum*
16	*P. vivax*	*P. vivax*
17	*P. falciparum*	*P. falciparum*
18	*P. falciparum*	*P. falciparum*
19	*P. falciparum*	*P. falciparum*
20	*P. vivax*	*P. vivax*
21	Neg	*P. malariae*
22	*P. malariae*	*P. malariae*
23	Neg	Neg
24	*P. falciparum*	*P. falciparum*
25	*P. falciparum*	*P. falciparum*
26	*P. malariae*	*P. malariae/P. ovale*
27	*P. ovale/P. malariae*	*P. malariae*
28	*P. falciparum*	*P. falciparum*
29	Neg	Neg
30	*P. vivax*	*P. vivax*

DISCUSSION

The analysis of malaria parasite resulted in positive detection of 28 samples out of the 30 samples analyzed. Two samples resulted negative, both microscopy and PCR diagnosis, one sample was diagnosed negative by the means of microscopy analysis, but PCR based diagnosis revealed *P. malariae* infection. Failure to detect *P. falciparum, P. vivax and P. vivax* was observed in three cases on microscopy diagnosis and two cases of *P. ovale* and one case of *P. malariae* was observed on PCR diagnosis.

CONCLUSION

PCR based techniques was developed to detect low level parasitaemia, allows species identification, detect mixed infection, therefore can be used as an excellent tool for diagnosis of malaria parasites when conventional techniques show negative results.

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