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Testing of newly developed glycophospholipid antigen for the detection of *P. falciparum* malaria by laser light immunoassay in endemic and non-endemic areas

A. Roy[a*], S. Biswas[a], M.M. Mya[b], R.K. Saxena[b] & K.B. Roy[c]

[a]*Malaria Research Centre(ICMR), 22 Sham Nath Marg, Delhi;* [b]*Centre for Biomedical Engineering, Indian Institute of Technology, New Delhi;* [c]*Centre for Biotechnology, Jawaharlal Nehru University, New Delhi, India*

A glycophospholipid (GPL) antigen isolated from *Plasmodium falciparum* culture supernatant has been tested for its antigenicity. Detection of malaria positive known blood samples and unknown field samples from endemic and non-endemic areas were compared. In this study laser light scattering immunoassay (LIA) was used for the detection of *P. falciparum* malaria. Test results of control (malaria negative samples from Surat) were compared with known positive samples and unknown malaria positive field samples. A positive correlation has been observed (97%) in falciparum positive samples from laboratory and unknown samples from endemic area (Haldwani) by LIA method using GPL antigen. From the results of the study it was found that GPL antigen has a better antigenic property and can detect almost all the cases of *Pf* malaria by LIA method.

Key words Laser light immunoassay – microscopic examination – per cent positivity – *Pf* malaria

Plasmodium falciparum (*Pf*) malaria is a common communicable disease of third world countries and causes high morbidity and mortality. The disease can be controlled if it is diagnosed and treated at an early stage of onset. Several methods for the diagnosis of malaria are available and the most common method is microscopic examination of blood smears. Although microscopic method provides a reliable diagnosis of the disease but some times due to poor quality of blood smears the results may not be accurate and it also depends on expertise and diligence of technicians. Several other diagnostic techniques for malaria have been developed such as fluorescent dye technique, buffy coat technique, Kamamoto fluorochrome technique etc[1,2]. All these methods have certain limitations as that of light microscopy.

**Corresponding author*

Among immunological methods the enzyme linked immunoabsorbent assay (ELISA) has been found to be quite sensitive[2,3]. Several other antigen and monoclonal antibody based methods for the diagnosis of malaria have been developed such as Immunochromatographic test[4], ParaSight 'F' test[5], OptiMAL assay[6] and rapid dipstick antigen capture assay[7], but all these immunological methods are expensive and beyond the reach of poor developing countries where malaria is highly endemic. Methods based on the identification of parasite specific nucleic acids by use of the polymerase chain reaction[8-11] have been used for parasite detection. Unfortunately all these methods are costly, needs skilled technical manpower and sophisticated laboratory. Therefore, it is felt that there is still a need to develop a simple, rapid and economical method for diagnosis of malaria. Recently, laser light scattering immunoassay

(LIA) method has been reported to be very sensitive technique for the detection of *Pf* malaria[12] and has been found more sensitive than enzyme immunoassay. LIA technique for the detection of *Pf* malaria is a simple method and can diagnose 95% of all *Pf* cases. Several antigens had been used for the detection of malaria but none had been found to provide very high rate of immunoreactivity with positive blood samples. We have isolated and purified a new kind of *Pf* antigen—glycophospholipid (GPL) (inositol free) from *in vitro* culture supernatant of *P. falciparum*. An antigenic property of GPL has been evaluated by LIA method using different known positive blood samples.

Material & Methods

Preparation of glycophospholipid (GPL) antigen from Pf culture supernatant : This antigen was isolated from *P. falciparum in vitro* culture supernatant which was made free from erythrocyte and parasite debris by differential centrifugation, concentrated, dialysed, lyophilised and extracted with chloroform-methanol-water mixture according to our published protocol[13]. Chloroform extract was found out to be glycophospholipid. This antigen contains galactose, glucose, xylose and mannose. It is completely soluble in ether and chloroform. It is a sticky material, difficult to handle and its TLC mobility is slow indicating presence of lipid.

Study group

Malaria positive blood sample collection : Active fever case blood samples (50) were collected in September to October 1999 during transmission season from endemic area of Haldwani. Blood samples were collected with the informed free consent of the patients and stored on filter paper.

Negative control blood collection : Humans subjects aged between 20 and 40 years of both sexes were selected after careful medical examinations and those having no history of malaria were used as negative control. Blood samples negative for malaria (negative control) were collected from Surat. Finger prick blood was collected on filter paper. Requisite approval was taken for collection of blood from the donors. Blood samples on filter paper were stored at 20°C in deep freezer.

Testing of blood samples by LIA

Preparation of antigen coated latex beads for LIA study : 25 µl of 1 mg/ml of the antigen solution and 1 ml of 1% latex beads in carbonate-bicarbonate buffer (pH 9.6) were mixed and kept over night at 4°C. Later, latex bead suspension was incubated at 37°C for one hour in an incubator. Then the bead suspension was washed three times with distilled water and collected by centrifugation at 20,000 rpm at 25°C for 30 min. Washed beads were resuspended in 100 µl of 1% BSA (bovine serum albumin) solution . Suspended beads were reincubated for one hour at 37°C. After incubation the beads were washed again three times with distilled water and centrifuged at 20,000 rpm at 25°C for 30 min. After final washing, the beads were suspended in 1 ml carbonate-bicarbonate buffer (pH 9.6) and kept at 4°C. 18 µl of antigen coated latex beads and 2 µl of 1:100 diluted blood samples [2 punched discs (5 µl blood in each) from filter paper was eluted in 0.5 ml 0.1M PBS (pH 7.2)] was thoroughly mixed in a 1.5 ml eppendorf tube. The mixture was then immediately drawn into a 6 cm long and 3 mm diameter glass capillary tube so that the mixture stays in the lower one-third part of the capillary tube. Both ends of the tube were closed with paraffin wax and kept for one hour at room temperature. After one-hour, sample capillary tube was placed in the sample holder of LIA. LIA measurements of blood samples were done as per published procedure[14].

Study of blood samples from endemic and non-endemic field areas by LIA method

Study subjects

In laboratory : About 50 finger prick blood samples were collected during transmission season from villag-

es Shaulana and Lalpur, District Ghaziabad in the years 2000 and 2001 on filter papers from microscopically identified *Pf* patients and 50 samples from healthy individuals as negative control. Sample blood was eluted from filter paper with PBS buffer just before laboratory testing. *P. falciparum* specific antigen molecules like GPL1[13] were used and studied by LIA techniques and their results were compared with that of standard microscopic method.

Control group : Blood samples were collected from healthy donors (Surat, Gujarat) and served as negative controls. About 50 samples of finger prick blood were collected on 3 mm thick Whatman filter paper. Thick and thin blood smears on glass slides were also prepared for microscopic examination to confirm the absence of malarial parasites.

Endemic area : About 50 active fever cases finger prick blood samples from Haldwani, District Nainital, Uttaranchal (identified endemic area) were collected on 3 mm thick Whatman filter paper for LIA study. Also thick and thin blood smears on slides were made for microscopic confirmation of the disease.

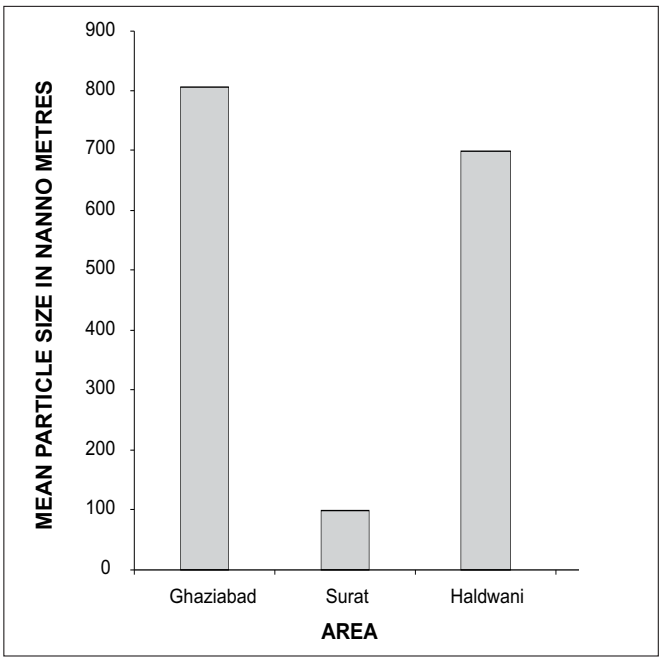

Fig. 1: P. falciparum positivity detected by laser light immunoassay

Results & Discussion

Antigenic sensitivity of isolated *P. falciparum* glycophospholipid, devoid of inositol has been measured by laser light immunoassay in blood samples of patients and healthy individuals to test its efficiency in detection of malaria parasite. Percentage positivity values of LIA were compared with the results of microscopy. The sensitivity of LIA on latex agglutination in terms of seropositivity has been calculated in terms of a cut-off value decided as mean value of control (healthy) sera +2 SD and found to be 96% with GPL when compared with microscopy in laboratory (Fig.1 & Table 1). Immunopositivity was calculated. Significance of values was calculated by student 't' test and compared with control for the determination of 'p' value. All comparisons of values showed $p < 0.0001$ in all the sets of measurements. Filter paper blood samples from field studied by LIA method showed 97.36% detection rate with GPL antigen, whereas samples of the control group were found negative. Highest sensi-

Table 1. Comparison of malaria positivity by microscopy and laser light immunoassay

Microscopic examination	LIA of laboratory samples	LIA of field samples	
		Surat (control, n= 50)	Haldwani (*Pf* 38/50)
Pf +ve (50)	*Pf* + ve 96% (48)	0 (0)	37 (97.36)
Mean + SD	804.96 + 182.23	98.7143 + 3.8527	699.047 (237.309)
p-value	< 0.0001	< 0.0001	

Figures in parentheses indicate samples detected positive; Cut-off value —106.

tivity for detection of malaria was seen with LIA and results are compared with earlier results described elsewhere[12,14]. Blood samples showed that LIA with GPL antigen is a sensitive and reliable method for the detection of falciparum malaria. Present study provides information on the antigenicity of GPL and its use in detection of *Pf* infected blood by LIA method.

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