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ROLE OF pLDH ANTIGEN AS NON MICROSCOPIC IMMUNOLOGICAL MARKER IN DIAGNOSIS OF MALARIAL PARASITE

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ABSTRACT

Background: Malaria is a serious condition and prognosis depends on timely diagnosis. Although microscopy remains the cornerstone of diagnosis, plasmodium LDH antigen detection test are increasingly used. They are easy to use, provide results rapidly and require no specific training and equipment. Reported sensitivities vary but are generally good for Plasmodium falciparum. **Methods: -** total 100 cases were taken. Thick and thin smear are prepared using 10% guess stain and examined under microscope. Malaria test done by pLDH dipstick method based on immune-chromatography. **Results: -** comparing pLDH antigen detection test with microscopy the sensitivity is 85.42% and specificity is 100%. Specificity for P. falciparum is 58% and for P. vivax is 22%. **Conclusion: -** These tests have several limitations, including cross-reactions of P. falciparum with the non-falciparum test line and vice versa and (rare) false-positive reactions due to other infectious agents or immunological factors. In the diagnostic laboratory, dipstick methods are a valuable adjunct to (but not a replacement for) microscopy for the diagnosis of malaria.

Keyword: pLDH antigen, immunochromatography, malaria.

INTRODUCTION

Peer Malaria is the world's most common tropical disease after tuberculosis **(1)**. It affects more than 2400 million people i.e. 40% of world's population. Worldwide prevalence is estimated around 300-500 million each year **(2)**. In India, entire population is now deemed to be under malaria risk **(3, 4)**. Gujarat has 6.6% of total cases and 3.9% of P.falciparum cases **(5)**.

The causative agents in humans are 4 species of plasmodium protozoa (single celled parasites) P falciparum. P. vivax, P. Ovale P. malariae. Of these P.falciparum accounts for majority of infections and is the most lethal **(5, 6, 7)**.

Being associated with most serious complications, diagnosis of malaria constitutes a medical emergency. Generally treatment is based on clinical symptoms. But the emergency of Chloroquine resistant malaria has made it urgent to ensure that treatment is base on rapid and reliable diagnosis of disease **(8)**.

For decades, light microscopy of blood smears has been the gold standard for malaria diagnosis. But now numerous new diagnostic techniques have been developed **(9)**.

pLDH antigen detection tests have ability to rapid diagnosis with differentiation of P.falciparum and P.vivax. It utilizes a dipstick coated with monoclonal antibodies against intracellular metabolic enzyme parasite LDH. Differentiation of malaria parasite is based on antigenic differences between pLDH isoforms. It also has the ability to distinguish viable from non-viable parasites, appears to be promising tool for monitoring therapy with antimalarial drugs. They are commercially available kits, which include all necessary reagents and do not require extensive training or equipment to perform or interpret their results **(8)**.

METHODOLOGY:

Total 100 cases clinically personated as malaria in medicine & pediatric wards of SSGH Barod were taken. Thick & thin smear prepared by using 25 mm x 75 mm glass slide. Staining was done by 10% Giemsa stain, Giemsa Stock solution (5 ml) was diluted in 100 ml of buffer water pH 7.2.

Film was fixed with high alcohol for 30 seconds and kept for 30 to 40 minutes. Later slides were flushed with tap water gently & were left to dry themselves. Films were examined under oil immersion. The only elements are parasites, leucocytes & faint out line of RBC. Young trophozoites appear as broken rings & spots of blue cytoplasm with detached chromatin dots.

A rough estimate of parasite concentration is estimated by counting every number of parasites observed per oil immersions field of thick film as follows.

Malaria test was done by pLDH dipstick method. It is an Immuno chromatographic tests based on the capture of parasite antigen from peripheral blood using monoclonal antibodies prepared against a malaria antigen target & conjugated to other a liposome containing selenium dye or gold particles in a mobile polar **(8,10,11,12)**.

Test results

1. Positive

P. Falciparum or mixed infection - 2 solid pink lines Control pink dash

P. Viva, P. ovale, P. malariae - 1 Solid pink line Control pink dash

2. Negative

- No pink line & Control pink dash

3. Unpredictable

- No pink line & No pink dash (control)

- repeat the test

RESULT:

This is a study of sensitivity and specificity of pLDH antigen detection test in the malaria cases. Total 100 cases with clinical presentation of malaria are taken.

Clinically susceptible cases were distributed according to age and sex (Table 1).

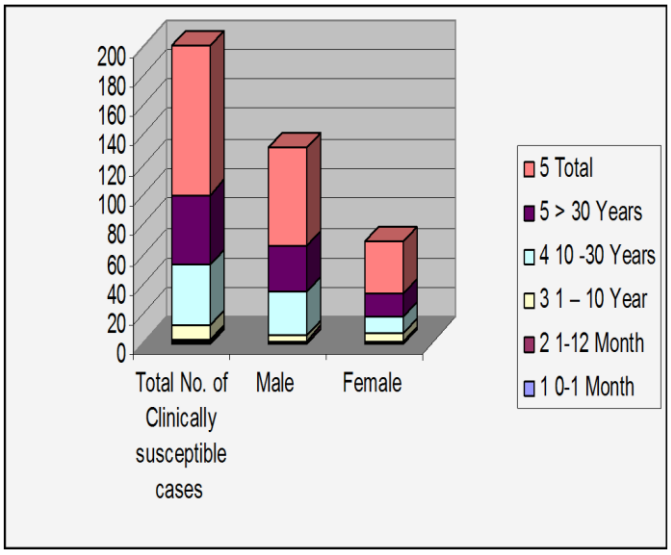
The most common age group in present study in above 30 years and there is male preponderances.

The table 2 shows, among 100 clinically suspected cases 80% are present by dipstick method.

Table 3 shows, by dipstick method among 100 clinically suspected cases 58% are P. falciparum positive while 22% are P. vivax positive.

Table-1 Age and sex wise distribution of clinically susceptible cases

Sr. No.	Age Group	Total No. of Clinically susceptible cases	Male	Female
1.	0-1 Month	01	0	**01**
2.	1-12 Month	02	02	**0**
3.	1 – 10 Year	09	04	**06**
4.	10 -30 Years	41	29	**11**
5.	> 30 Years	47	31	**16**
	Total	**100**	**66**	**34**



pLDH ANTIGEN DETECTION TEST

Table-2 Shows results of pLDH antigen detection test.

Sr. No.	Cases Examined	Dipstick positive	Dipstick Negative
1.	Group A : 100	82	**18**
2.	**Group B: 40**	**36**	**04**

Table-3 specification of malaria parasite by dipstick method.

	P. falciparum	P. Vivax	Mixed	Negative	Total
Cases	**58**	**22**	**02**	**18**	**100**

Table -4 Comparison of dipstick with microscopy

	No. of positive cases by Microscopy	No. of Positive cases by Dipstick	No. of Negative case by Dipstick
P. falciparum	67	58	**09**
P. Vivax	27	22	**05**
Mixed	02	02	**00**
Total	**96**	**82**	**14**

In above study, the microscopy examination is taken as gold standard.

CALCULATION

pLDH	**Microscopy**		
	POSITIVE	NEGATIVE	**TOTAL**
POSITIVE	82(a)	00 (b)	**82**
NEGATIVE	18 (c)	04	**18**
Total	**96**	**04**	**100**

DISCUSSION:

Malaria is a global health problem which increases worldwide. Improvement in malaria diagnostic should facilitate in identification of individuals suspected with the malaria.

Newer more advanced malaria diagnostics based on fluorescent microscopy and detecting of nucleic acid (PCR) are well known, but there are limitations too. Recently introduced diagnostic test based on immunocapture assays solve this problem **(13)**.

During the present study 100 cases were analyzed in respect of clinical presentation by routine microscopic methods and the immunocaputre assay techniques namely pLDH antigen detection for rapid P.falciparum and P. vivax detection .In our study, we observed male preponderance as far as the sex is concerned and is comparable with the study carried out at Malaria Research Center, Civil hospital, Nadiad (Gujarat) **(14)**.

The positive predictive value correlated with the blood peripheral examination (96%) along with the positive serological test (82%) and this can be compared well with the hospital attended and diagnosed case analysis carried out by Basu et al, at Calcutta **(15)**.

Though the clinical picture of malaria to differentiate the two different parasites viz . P.vivax and P. falciparum, at times is confusing; but with the careful thick and thin peripheral blood smear examination made it easy. With the fact that, it was correlated well with serological maker i.e. pLDH antigen detection . Our case-study showed 67% positivity rate for P. falciparum blood smear. The pLDH antigen detection was positive in 58% of P. falciparum cases while 22% of P.vivax cases were positive by same technique. These results are compared well with the case analysis carried out at Nanda Hemvani at Clorithram Hospital and research Center, Indore **(16)**.

The present study evaluates the comparison of methodology used for definite diagnosis of specific parasite by conventional method such as the thick and thin blood smear examination with the serological marker viz. pLDH antigen detection immunocaptureassay which gives 100% specificity and 85.42% sensitivity along with its other merits explained earlier and documente **(16)**.

CONCLUSION

Total 100 cases clinically presented as malaria in medicine and pediatric wards of NCH, SURAT were subjected to rapid pLDH antigen detection test for diagnosis of malaria to establish sensitivity and specificity of the method. Smear examination is taken as standard .The maximum incidence is in above 30 years age group. Male to female ratio is found 1.94:1 .Among clinically suspected 100 cases 96% showed blood smear positivity, so sensitivity of clinically suspected cases is 96%. Among all smear positive P. falciparum is found in 67% and P. vivax in 27%. Comparing pLDH antigen detection test with

microscopy the sensitivity is 85.42% and specificity of test is 100%. The species specificity of this test for P. falciparum is 86.57% and P. vivax is 81.48%.

Conflict of interest: Nil

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