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PREVALENCE AND COMPARISON OF DIFFERENT DIAGNOSTIC METHODS IN DIAGNOSIS OF MALARIA.

Medical Microbiology

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ABSTRACT

Background: Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected female Anopheles mosquitoes. It is preventable and curable. In 2019, there were an estimated 229 million cases of malaria worldwide.
Material And Method: 100 samples were included in the study, which further processed for QBC, rapid card and peripheral blood smear diagnostic test for diagnosis of malaria.
Result And Observation: Out of total 100 cases thick smear detected 73 positives compared to 70 by thin smear, 72 by dipstick, and 69 by QBC. Thick smear proved to be better than the other methods. While dipstick closely followed thick smear, thin smear and QBC had a back stage in comparison.
Conclusion: Thick smear proved to be better than the other methods. QBC hadthe disadvantage of difficulty in species identification.

KEYWORDS

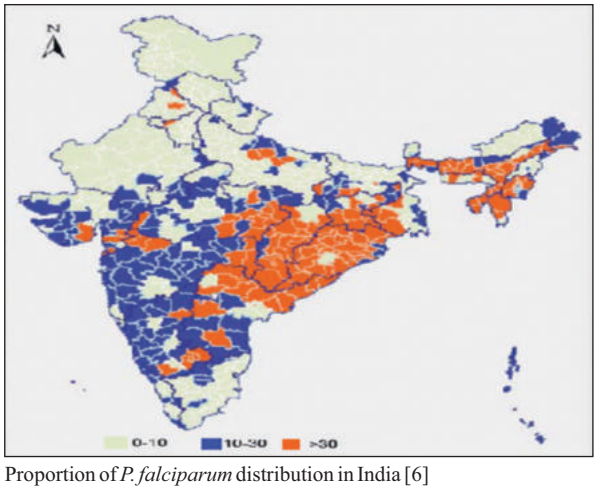
PBS, QBC, Dipstick, Malaria

INTRODUCTION: -

Malaria endemic areas in India: Malaria has been a problem in India for centuries. Details of this disease can be found even in the ancient Indian medical literature like the *Atharva Veda* and *CharakaSamhita*. In the 30's there was no aspect of life in the country that was not affected by malaria. During the latter parts of nineteenth and early twentieth centuries, nearly one-fourth of India's population suffered from malaria, particularly in the states like Punjab and Bengal.[1] The economic loss due to the loss of man-days due to malaria was estimated to be at Rs. 10,000 million per year in 1935.

At the time of independence in 1947, of a population of 330 million, about 75 million people were estimated to be infected with malaria every year, and the direct mortality due to the disease was estimated at 0.8 million per annum.[2,3] To combat this menace, the Govt. of India launched the National Malaria Control Programme in April 1953. The programme proved highly successful and the number of malaria cases significantly declined to about 2 million by 1958.[4] Encouraged by this, the programme was changed to a more ambitious National Malaria Eradication Programme in 1958. By 1961 the incidence dropped further to a mere 49151 cases, with no deaths.[5]

- In 2019, there were an estimated 229 million cases of malaria worldwide.
- The estimated number of malaria deaths stood at 409 000 in 2019.
- Children aged under 5 years are the most vulnerable group affected by malaria; in 2019, they accounted for 67% (274 000) of all malaria deaths worldwide.
- The WHO African Region carries a disproportionately high share of the global malaria burden. In 2019, the region was home to 94% of malaria cases and deaths.



Proportion of *P. falciparum* distribution in India [6]

Malaria, at one time a rural disease, diversified under the pressure of developments into various ecotypes. These ecotypes have been identified as forest malaria, urban malaria, rural malaria, industrial malaria, border malaria and migration malaria; the latter cutting across boundaries of various epidemiological types. Further, malaria in the 1990s has returned with new features not witnessed during the pre-eradication days. These are the vector resistance to insecticide(s); pronounced exophilic vector behaviour; extensive vector breeding grounds created principally by the water resource development projects, urbanization and industrialization; change in parasite formula in favour of *P. falciparum*; resistance in *P. falciparum* to chloroquine and other anti-malarial drugs; and human resistance to chemical control of vectors.[6]

MATERIALAND METHOD:

Study Design:
The present study is carried out in this institutein 2019-2021.

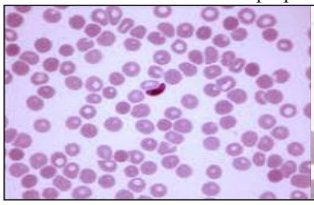
Specimen:
A total of 100 blood samples of suspected cases of malaria were collected from patients attending the outpatient department and admitted in medical wards.

Collection Of Specimen:
Capillary blood by finger prick method and/or venous blood collected in the EDTA bulb.

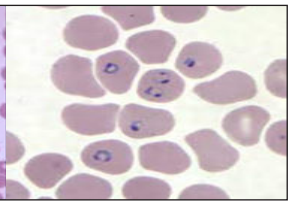
Capillary Blood Method:
By using sterile technique, the lobe of finger is cleaned using a swab moistened with spirit and the area is allowed to dry. With help of a sterile lancet, the finger is pricket and squeezed gently to obtain enough blood.

Venous Blood Method:
By using aseptic precautions, tourniquet is tied over the arm. The area of cubital fossa or any other site is cleaned with spirit and allowed to dry. About 2-3 ml of blood is collected in EDTA bulb with the help of 5 ml disposable syringe and 23 mm gauge needle. The bulb is gently shaken to mix the blood with anticoagulant.

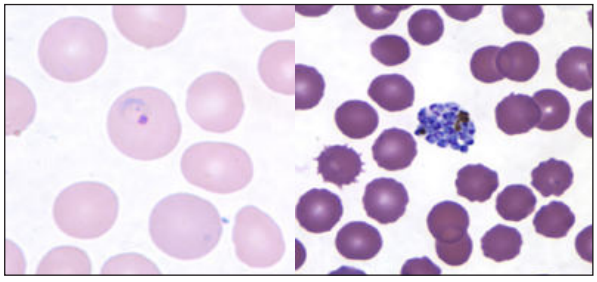
PS for MP:
Both thin and thick smears are prepared.



Gametocyte of P.falciparum



Ring forms of P.falciparum



Ring form of P.vivax SchizontofP.vivax

Procedure:
Every sample is tested by the following 3 methods:
P/S thin and thick smear.
Dip Stick Malaria—Optimal
QBC capillary method.

Thin Film Field's Staining Technique:
1. Place the slide on a staining rack and coverthe methanol fixed thin film with approximately 0.5 ml of diluted Field's Stain B.
2. Add immediately an equal volume of Field's stain. A and mix with the diluted Field's stain B. Leave to stain for 1minute.
3. Wash off the stain with clean water. Wipe the back of the slide clean and place it in a draining rack for the film to air dry.

Results For Malaria Thin Film:
Chromatin of parasite are appear darkred
Cytoplasm of parasite are appear blue
Schüffner's dots are appear redMauer'sdots(clefts) are appear red mauve
Malaria pigment in white cells are appear brown black Red
Red cells are appear grey to pale mauve pink
Reticulocytes are appear grey blue
Nuclei of neutrophils are appear dark purple
Cytoplasm of mononuclear Cellsare appearblue gray
Granules of eosinophils are appear red

Thick Film Field's Straining Technique
1. Holding the slide with the dried thick film facing downwards, dip the slide in to Field's Stain A for 5 sec. Drain off the excess stain by touching a corner of the slide against the side of the container.
2. Wash gently for about 5seconds in clean water. Drain off the excess water.
3. Dip the slide into Field's Stain B for 3 seconds. Drain off the excess stain.
4. Wash gently in clean water. Wipe the back of the slide clean and placeIt upright in a draining rack for the film to air dry.

Note: If after staining, the whole of the film appears yellow brown (usually a sign that too much blood has been used), too blue or too pink, do not attempt to examine it. Restrain it. (Dipping in field's stain A for 1 second, field's stain B for 1second).

Results For Malaria Thick Film:

S.N.	Characteristics of malaria parasite	color
1.	Chromatin	Dark red
2.	Cytoplasm	blue mauve
3.	Schüffner's dots	
4.	P.Vivax	Pale red
5.	P.ovale	Pale red
6.	Malaria pigment	Yellow brown orBlack brown
7.	Nuclei of small lymphocytes	Darkpurple
8.	Nuclei of neutrophils	DarkPurple
9.	Granules of eosinophils	Red
10.	Cytoplasm of mononuclear cells	Bluegrey
11.	Reticulumofreticulocytes	blue grey stippling

RESULTSAND OBSERVATION:
Table-1 shows age wise distribution of positive and negative cases of malarial. It shows that the occurrence of malaria is Maximum in 10 – 19, 20 – 29, and 60 and above age in both males and females. Out of 100 patients sampled 20 were in the age group 20-29 years and 19 each in the age group10 -19 and 60 & above years.

Table – 1 Age Wise Distribution Of Positive And Negative Cases

Age	Positive Male	Negative Male	Positive Female	Negative Female	Total Positive	Total Negative
0-9	2	3	1	2	3	5
10-19	7	2	8	2	15	4
20-29	8	3	8	1	16	4
30-39	6	1	3	3	9	4
40-49	4	1	3	3	7	4
50-59	4	2	2	2	6	4
60 AND ABOVE	13	1	5		18	1
Total	44	13	30	13	74	26

Table-2 shows age wise distribution of P. vivax, P. falciparum and mixed infections. All the 3 shows more occurrences in the 10-29 and 60 & above age. P. vivax and P. falciparum both had maximum occurrence in the age group10 to 29.

Table – 2 Age Wise Distribution Of P. Vivax, P. Falciparum And Mixed Infections

AGE	P vivax	P falciparum	Mix	Negative	Total
0-9	1	2	-	5	8
10-19	2	11	2	4	19
20-29	1	13	2	4	20
30-39	2	7	-	4	13
40-49	1	6	-	4	11
50-59	1	5	-	4	10
60 AND ABOVE	2	15	1	1	19
	10	59	5	26	100

DISCUSSION AND CONCLUSION
The present study and various other studies bring out various facts about diagnosis of malaria. Peripheral smear has stood the test of time and remains the Gold Standard in diagnosis of malaria.

Out of thin and thick smears, thick smear can identify more number of positive cases as larger volume of blood is taken compared to thin smear. Thus its sensitivity is higher than other methods.

Although thick smear has higher sensitivity, inappropriate method can lead to deformation of the morphology of parasite causing difficulty in species identification. Debris and other cellular material can also confuse with rings. Schizonts of P. vivax and gametes of P. falciparum may deform creating confusion. But if done properly, thick smear is the best method. Field stain consuming lesser time than Giemsa stain is preferable for rapid diagnosis.

Thin smear, as it takes lesser quantity of blood to make, may miss outifparasitemiais low. However a thin smear prepared properly gives a beautiful picture of rings inside RBCs, gametes-male and female and schizontsof P. vivax. After all nothing could be as original as seeing the malarial parasite in sideits normal habitat i.e .RBC.Dip stick has proved to be a good rapid method which can be used inlieu of peripheral smear in fields for mass survey, in laboratories where technical expertise is not available and to assess response to treatment. The density of colourof positive band is directly proportional to the degree ofparasitemia.

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