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Original Research Article.

A Comparative Study of Microscopic Detection Methods and Haematological Changes in Malaria

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ABSTRACT

Objective: To find out the relative incidence of different species of Plasmodium, most sensitive method for detection and various hematological changes in Malaria.

Methods: In 6 months study, blood sample were collected from 100 patients attending the OPD Laboratory in SMS Hospital, who were having malaria. Complete blood count and peripheral blood film examination (Thin and thick Leishman stained films), Quantitative Buffy Coat (QBC) test, and acridine orange fluorescence method were performed. Measurement of prothrombin time and partial thromboplastin time done by automated coagulorneter Dimed (CD-2). Determinaton of fibrin degradation products done in patients having prolonged prothrombin time and partial thromboplastin time by commercially available kit.

Results: Over the period of 6 months total 100 cases were analysed.on examination of thin film P. falciparum 78% cases and P. vivax 22% cases.on thick film examination P. falciparum 61% cases and P. vivax 21% cases. In QBC test 45% cases P. falciparum and 7% cases P vivax. In acridine orange fluorescence method 51% cases P. falciparum and 17% cases P. vivax. Out of 100 malaria positive cases, malaria was detected by thin smear examination in 100% cases, by thick smear examination in 82% cases, by acridine orange fluorescence method in 68% cases while QBC has least sensitivity as only 52% cases were detected by this method. Anemia was present in 78.20% cases of P. falciparum and 54.5% cases of P. vivax. Thrombocytopenia was present in 87.17% cases of P. falciparum and 54.54% cases of P. vivax. In P. falciparum PT was found within normal range in 89.7% cases and prolonged in 10.2% cases. APTT was normal in 87.1% cases and prolonged in 12.8% cases. In P. vivax, PT was found normal in 100% cases and APTT was found within normal range in 95.4% cases and prolonged in 4.5% cases. Out of 100 cases, FDP was positive in 6% cases of malaria and all the cases were of P. falciparum.

Conclusion: Malaria has been a problem in India for centuries. There is always a need for a rapid and sensitive method for identifying malarial parasite. Prompt and accurate diagnosis of malaria is important for effective cases management.Thin film examination was found to be the most sensitive method while QBC test was least sensitive.

Key words: Malaria, QBC, Acridine Orange.

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INTRODUCTION

Malaria is one of the major public health problem in the developing countries. Recent estimates indicate that annually between 300-500 million clinical cases and between 1.5-2.7 million deaths occurs due to malaria worldwide.[1]

Malaria ranks 3rd among the major infectious disease in causing death after pneumococcal acute respiratory infection and tuberculosis. Malaria would be the no. 1 infectious killer disease in the world.[2] Because of such a high incidence of malaria in India and reasonably high incidence of complications of malaria especially cerebral malaria in paediatric and pregnant patients.

There is always a need for a rapid and sensitive method for identifying malarial parasite and various researchers have in time to time tried to compare all the methods and study the efficacy of each method.[3]

AIMS & OBJECTIVES

- To find out the relative incidence of different species of Plasmodium.
- To find out which method of detection is more sensitive.
- To study the changes in haemogram according to species.

- To study the platelet count according to species.
- To study the coagulation profile mainly prothrombin time and activated partial thromboplastin time.
- To study the incidence of fibrin degradation product in patients having prolonged PT and APTT.

MATERIALS & METHODS

The present study was conducted in the Department of Pathology, SMS Medical College and associated group of Hospitals, Jaipur.

The blood samples were collected from 100 patients attending the OPD Laboratory in SMS Hospital, who were having malaria.

Complete blood count and peripheral blood film examination (Thin and thick Leishman stained films), Quantitative Buffy Coat (QBC) test, and acridine orange fluorescence method were performed over a period of 6 months.

Measurement of prothrombin time and partial thromboplastin time done by automated coagulorneter Dimed (CD-2). Determinaton of fibrin degradation products done in patients having prolonged prothrombin time and partial thromboplastin time by commercially available kit.

Table 1: Relative incidence of different species of Plasmodium in 100 patients of malaria in Leishman stained thin film examination

Species of Plasmodium	No. of cases	%
Plasmodium falciparum	78	78%
Plasmodium vivax	22	22%
Plasmodium malariae	0	0%
Plasmodium ovale	0	0%

Table 2: **Relative incidence of different species of Plasmodium in 100 patients of malaria in Leishman stained thick film examination**

Species of Plasmodium	No. of cases	%
Plasmodium falciparum	61	61%
Plasmodium vivax	21	21%
Plasmodium malariae	0	0%
Plasmodium ovale	0	0%

Table 3: **Relative incidence of different species of Plasmodium in 100 patients of malaria in Quantitative Buffy Coat (QBC) test**

Species of Plasmodium	No. of cases	%
Plasmodium falciparum	45	45%
Plasmodium vivax	7	7%
Plasmodium malariae	0	0%
Plasmodium ovale	0	0%

Table 4: **Relative incidence of different species of Plasmodium in 100 patients of malaria in acridine orange fluorescence method**

Species of Plasmodium	No. of cases	%
Plasmodium falciparum	51	51%
Plasmodium vivax	17	17%
Plasmodium malariae	0	0%
Plasmodium ovale	0	0%

OBSERVATION AND RESULTS

Table No. 1 shows Relative Incidence of different species of Plasmodium on thin film examination. Number of cases positive for different Plasmodium species on Leishman stained thin film were as follows P. falciparum 78% and P. vivax 22%. Cases of P. malariae and P. ovale were Nil as these species are not found in Rajasthan.

Table No. 2 shows Relative Incidence of different species of Plasmodium on thick film examination. Number of cases positive for different Plasmodium species on Leishman stained thin film were P. falciparum 61% and P. vivax 21%. Cases of P. malariae and P. ovale were Nil as these species are not found in Rajasthan.

Table No. 3 shows Relative Incidence of different species of Plasmodium by QBC test. Number of cases positive for different Plasmodium species by QBC test. 45% P. falciparum and 7% P vivax .Cases of P. malariae and P. ovale were Nil as these species are not found in Rajasthan.

Table No. 4 shows Relative Incidence of different species of Plasmodiurn on acridine orange fluorescence method. Number of cases positive for different Plasmodium species on in acridine orange fluorescence method were 51% P. falciparum and 17% P. vivax Cases of P. malariae and P. ovate were Nil as these species are not found in Rajasthan**.**

Table no. 5 shows Sensitivity of different methods of diagnosis in 100 malaria positive cases. Out of 100 malaria positive cases, malaria was detected by thin smear examination in 100% cases, by thick smear examination in 82% cases, by acridine orange fluorescence method in 68% cases while QBC has least sensitivity as only 52% cases were detected by this method.

Table no. 6 shows sensitivity of different method of diagnosis according to species of Plasmodium. In Leishman stained thin smear examination, 78% cases of P. falciparum and 22% cases of P. vivax were diagnosed. In Leishman stained thick smear examination, 61% cases of P. falciparum and 21% cases of P. vivax were diagnosed. In QBC test. 45% cases of P. falciparum and 7% cases of P. vivax were diagnosed. In Acridine orange fluorescence method, 51% cases of P. falciparum and 17% cases of P. vivax were diagnosed. Cases of P. malariae and P. ovale was Nil as these species are not found in Rajasthan.

Table no. 7 shows value of haemogram according to species of Plasmodium. Anemia was present in 78.20% cases of P. falciparum and 54.5% cases of P. vivax. The anemia is more common in P. falciparum than P. vivax. In P. falciparum malaria 12.82% cases had mild anemia, 30.76% cases had moderate anemia and 34.61% cases had severe anemia. In P. vivax malaria, mild anemia was present in 18.18% cases, moderate anemia in 27.27% cases and severe anemia in 9.09% cases.

Table no. 8 shows value of total leucocyte count according to Species of Plasmodium. Total leucocyte count was within normal range in 50% cases of P. falciparum, leucopenia in 17.94% cases and leucocytosis was found in 32.05% cases. In P. vivax malaria, normal leucocyte count was found in cases, leucopenia in 4.54% cases and leucocytosis in 4.54% cases.

Table no. 9 shows platelet value according to species of Plasmodium. Thrombocytopenia was present in 87.17% cases of P. falciparum and 54.54% cases of P. vivax.

Table No. 10 shows PT and APTT value according to species of Plasmodium. In P. falciparum PT was found within normal range

in 89.7% cases and prolonged in 10.2% cases. APTT was normal in 87.1% cases and prolonged in 12.8% cases. In P. vivax, PT was found normal in 100% cases and APTT was found within normal range in 95.4% cases and prolonged in 4.5% cases.

Table no. 11 shows presence of FDP in 100 cases of malaria. Out of 100 cases, FDP was positive in 6% cases of malaria and all the cases were of P. falciparum. Table no. 12 shows presence of FDP according to species of Plasmodium in patients having prolonged PT. In P. falciparurn 10.2% cases had prolonged PT and among them FDP was positive in 50% cases. In P. vivax PT was normal in 100% cases and FDP was not positive in any case.

Table no. 13 shows presence of FDP according to species of plasmodium in patients having prolonged APTT. In P. falciparum 12.8% cases had prolonged APTT and among them FDP was positive in 20% cases. In P. vivax malaria APTT was prolonged in 4.5 % cases and FDP was negative in this case.

Table 5: **Sensitivity of different methods of diagnosis in 100 malaria positive cases**

	Thick smear Examination	Thin smear examination	QBC	Acridine orange method
Positive	100	82	52	68
Negative	0	18	48	32

Table 6: Sensitivity of different methods of diagnosis in 100 malaria positive cases according to species

	Thick smear examination	Thin smear examination	QBC	Acridine orange method
Plasmodium falciparum	78	61	45	51
Plasmodium vivax	22	21	7	17
Plasmodium malariae	0	0	0	0
Plasmodium ovale	0	0	0	0

Table 7: Value of haemogram according to species in 100 patients of malaria

Species	Total No. of cases	Haemoglobin gm/dL							
		<6 gm/dL		6-9 gm/dL		9-11 gm/dL		>11 gm/dL	
		No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
P.Falciparum	**78**	27	34.61%	24	30.76%	10	12.82%	17	21.7%
P. Vivax	**22**	2	9.09%	6	27.27%	4	18.18%	10	45.4%

Table 8: **Value of total leucocyte count according to species in 100 patients of malaria**

Species	Total No. of cases	TOTAL LEUCOCYTE COUNT/mm^3					
		<4x10^3/mm^3		4-11x10^3/mm^3		>11x10^3/mm^3	
		No. of cases	%	No. of cases	%	No. of cases	%
P.Falciparum	**78**	14	17.94%	39	50%	25	32.05%
P. Vivax	**22**	1	4.54%	20	90.9%	1	4.54%

Table 9: **Platelet value according to species in 100 patients of malaria**

Species	Total No. of cases	PLATELET COUNT/mm^3							
		<50x10^3/mm^3		50-100x10^3 / mm^3		100-150x10^3 / mm^3		>150x10^3 / mm^3	
		No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
P.Falciparum	**78**	40	51.28%	22	28.20%	6	7.69%	10	12.8%
P. Vivax	**22**	2	9.09%	4	18.18%	6	27.27%	10	45.4%

Table 10: **PT and APTT value according to species in 100 patients of malaria**

Species	Total no. of cases	PT				APTT			
		Normal		Prolonged		Normal		Prolonged	
		No. of cases	%	No. of cases	%	No. of cases	%	No. of cases	%
P. falciparum	**78**	70	89.7%	8	10.2%	68	87.1%	10	12.8%
P. Vivax	**22**	22	100%	0	0%	21	95.4%	1	4.5%

Table 11: **FDP positive in 100 cases of malaria**

Species	Total no. of cases	FDP +ve cases
P. falciparum	78	6
P. Vivax	22	0
Total	100	6

Table 12: FDP according to species in patient having prolonged PT value in 100 patients of malaria

Species	Total no. of cases	Prolonged APTT	FDP positive
P. Falciparum	78	08	04
P.Vivax	22	0	0

Table 13: FDP according to species in patient having prolonged APTT value in 100 patients of malaria

Species	Total no. of cases	Prolonged APTT	FDP positive
P. Falciparum	78	10	02
P.Vivax	22	01	0

DISCUSSION

The present study was conducted in the Department of Pathology, SMS Medical College and associated group of hospitals, Jaipur.

The blood samples were collected from 100 patients attending the OPD Laboratory in SMS Hospital who were having malaria.

In present study out of 100 cases, 100% samples were Positive for malaria parasite by thin film examination, 82% samples were positive by thick film examination, 52% samples were positive by QBC technique and 68% samples were positive Acridine orange Fluroscence test. As compared to the study of Meenal Jain et al, of 200 sample studied, 25% samples by thin smear, 31 % samples by thick smear, 35% samples were positive by QBC technique. 4% samples were detected by QBC technique alone, had low parasite index.

Krishna BVS et al in the study found that out of 1435 samples examined, 57 patients were diagnosed to have malaria. 57 patients were diagnosed to have malaria. The malarial parasites were detected in both thick blood film and QBC method in 44 (77.19%) cases while in 13 (12.81%) cases the parasites were detected by QBC alone. All the samples which 10 were negative by QBC method were also negative by Leishman stained blood film examination.[4]

In our study thin film examination is best, easy, simplest, most sensitive and cost effective method for diagnosis of malaria. It is best for identification of species of Plasmodium and also show associated haematological changes (Makler et al, 1998).

In present study the incidence of various P. species showed that out of 100 cases, P. falciparum was detected in 78% cases in P. Vivax in 22% which is almost similar to the study carried by Amar Taksande et al who found P. falciparum in 72.7% cases and P. vivax in 22.7% cases.

Beale PJ et al in the study showed that 72.7 cases had malaria due to P. Vivax and 24.24% cases due to P. falciparum .[5]

Kelton John et al in the study found P. Vivax in 92.85% cases to P. falciparum in 7.14% cases.[6]

In our study 78% cases were of P. Falciparum and 22% cases were of P. Vivax. The reason for this high incidence of P. Falciparum may be that this study is done at SMS medical college, Jaipur which is a tertiary referred center where most of the complicated cases are referred from periphery and the complications are more common in P. falciparum malaria.

In present study anemia was present in 78.20% cases of P. falciparum and 54.50% cases of P. Vivax. Sharma SK Das et al found anemia in 86.7% patients of P. Falciparum.[7]

In present study normal leucocyte count was found in 50% cases, leucopenia in 17.94% cases and leucocytosis in 32.05% cases of P. falciparum. In P. vivax malaria cases normal leucocyte count was found in 90.9% cases, leucopenia in 4.54% cases and leucocytosis in 4.54% cases.

M.K. Mohapatra et al found normal leucocyte count in 76% cases, leucopenia in 3.3% cases and leucocytosis in 20% cases of P. falciparum and 100% cases of P. vivax showed normal leucocyte count.[8]

In the present study, thrombocytopenia found in 87.17% cases of P. falciparum and 54.54% cases of P. vivax.

Sharma S.K. Das el al also observed thrombocytopenia in 39.3% cases of P. Falciparum.[7] Anjum Parvez et al found thrombocytopenia in 39.3% cases of P. Falciparum.[9]

In the present study prolonged PT was found to be prolonged in 8% cases and prolonged APTT in 11% cases.

The results are compared with the study of Beale P.J. et al who observed prolonged PT in 15.15% cases.[5]

Kelton John et al in the study concluded at department of Medicine and Pathology, McMaster University Canada observed prolonged APTT in 9.09% cases.[6]

In present study detection of FDP (Fibrin degradation products) was done in only those patients who had prolonged PT and APTT. Out of 100 cases, FDP was performed in 19 cases showing prolonged PT & APTT. Six cases were positive for FDP and all these cases were of P. falciparum and none were of P. vivax.

In present study FDP was positive in 6 out of 78(7.6% cases) cases of P. falciparum. In these patients prolonged PT and APTT was observed in 4(5.1%) and 2(2.6%) cases respectively.

M.K.Mohapatra found raised FDP in 5 out of 60(8.3%) cases of P. falciparum. prolonged PT & APTT was found in 2(3.3%) and 3(4.9%) cases respectively.[8]

SUMMARY & CONCLUSION

- Malaria has been a problem in India for centuries. There is always a need for a rapid and sensitive method for identifying malarial parasite.
- Prompt and accurate diagnosis of malaria is important for effective cases management.
- Thin film examination was found to be the most sensitive method while QBC test was least sensitive.
- QBC is technically difficult to perform and its chief drawback is its high cost.

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