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Comparative Study of Peripheral Blood Smear, Modified Centrifuged Blood Smear And Dipstick Method In Diagnosis of Malaria With Evaluation of Hematological Parameters

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Abstract: *Nonspecific clinical presentation of malaria leads to overtreatment in endemic areas and missing the diagnosis in low transmission areas. The Peripheral Blood smear is an established method in malaria diagnosis. However low sensitivity, cumbersome technique and need for specially trained persons for interpretation limits its usefulness. The antigen detection method is highly sensitive and specific method, however mixed cases have to be confirmed on smears and quantification of parasitemia cannot be done, also it involves high cost. This study aims to establish Modified Centrifuged Blood Smear (MCBS) as a sensitive, specific and cost effective technique in malaria diagnosis. Also we aim to detect if presence of certain haematological indices would increase the probability of malaria in patients with acute febrile illness. Presence of such an indicator would prompt a more diligent search for malarial parasite and prompt institution of specific therapy.*

Materials and methods: *328 patients with symptoms suspicious of malaria were taken as cases. 200 others with no clinical suspicion of malaria acted as controls. Venous blood was collected and subjected to three techniques for diagnosis of malaria; Peripheral Blood Smear examination (PBS), Modified Centrifuged Blood Smear (MCBS) and Antigen detection (dipstick method). The blood was also subjected to analysis of Haemoglobin (Hb) value, Total WBC count (WBC), Platelet count, Differential count (DC) and Red cell Distribution Width (RDW).*

Results: *Modified Centrifuged Blood Smear method (MCBS) improves the sensitivity of peripheral blood smear method without compromising on specificity. Out of the haematological parameters assessed in this study, thrombocytopenia and anaemia were found to be indicators of malaria.*

Conclusion: *Use of MCBS in place of PBS will eliminate a lot of false negatives; also it is cost effective and requires no special training. Thrombocytopenia and anaemia have a strong association with malaria. Presence of these parameters in a clinically suspicious case should alert the clinician for the presence of malaria.*

Keywords: *antigen detection, malaria diagnosis, modified centrifuged blood smear, sensitive method, thrombocytopenia*

I. Introduction

The earliest symptoms of malaria are very nonspecific and variable such as fever, headache, body ache, malaise, fatigue and abdominal discomfort. The nonspecific nature of the clinical presentation of malaria may lead to over-treatment of malaria in malaria endemic areas and missing the diagnosis of malaria in low-transmission areas. Therefore, precise laboratory diagnosis and species identification is very essential.

The laboratory diagnosis of malaria is done by different techniques such as the conventional thin and thick peripheral blood smears (PBS), concentration techniques such as centrifuged buffy coat smears (CBCS) and fluorescent (QBC) technique, serologic tests such as the detection of parasite-specific proteins' Rapid Diagnostic Tests (RDT) and Polymerase Chain Reaction (PCR). These techniques have their own advantages and disadvantages with respect to sensitivity, specificity, time consumption, cost effectiveness, ease of procedure etc. It would be of great help if MCBS (modified centrifuged blood smear), a technique that utilizes most of the advantages, while eliminating most of the disadvantages of the above techniques, is implemented [1]. Also a variety of haematological alterations like progressively increasing anaemia, thrombocytopenia, leucocytosis Page 2 of 12 and leukopenia and rarely Disseminated Intravascular Coagulation (DIC) have been reported in Plasmodium falciparum malaria [2]. The present study aims to detect if certain haematological indices would increase the probability of malaria in patients with acute febrile illness. Presence of such an indicator may heighten the suspicion for malaria, prompting a more diligent search for the malaria parasite, and prompt institution of specific therapy.

II. Aims And Objectives

2.1 To assess the feasibility of Modified Centrifuged Blood smear (MCBS) test in early detection of malaria parasite.
2.2 To compare the detection of malaria parasite in clinically suspected cases by methods such as PBS, MCBS and antigen detection (dipstick method).
2.3 To determine whether change in any of the haematological parameters in malaria can play a significant role in predicting presence of malaria parasite in clinically suspected cases.
2.4 To determine whether MCBS can be said to be a new technique that utilizes most of the advantages, while eliminating most of the disadvantages of the conventional techniques.

III. Materials And Methods

The present study was done in a tertiary care hospital during the period from November 2009 to November 2011. 328 patients with symptoms suspicious of malaria (fever with chills, headache and nausea and vomiting) were enrolled in the study. Blood from 200 other patients, who had no clinical suspicion of malaria, was also taken and they acted as controls. From each of the 328 patients, detailed clinical history including age, sex, presenting complaints was taken. 2 ml of venous blood was collected in an EDTA bulb, subjected to three techniques for diagnosis of malaria; Peripheral Blood Smear examination (PBS), Modified Centrifuged Blood Smear (MCBS) and Antigen detection (dipstick method). The MCBS method is described below. The patient was declared to be having malaria if he was positive by any one of the methods employed and was called a CASE. The blood was also subjected to analysis by a three-part haematology analyser and Hb value, total WBC count, platelet count and RDW were noted. Differential count was done by peripheral smear examination. Similarly, blood from 200 patients negative for malarial symptoms was subjected to assessment of the same haematological parameters and were called CONTROLS. They were tested for malaria by all the three methods as well.

Modified Centrifuged Blood Smear (Mcbs) Technique [FIGURE 1&2] The centrifuged buffy coat smear (CBCS) which has been used in the past involves collection of 2ml venous blood into anticoagulant bottles, filling the wintrobe's tube and centrifugation for 20-30 min, finally obtaining buffy coat layer onto a slide. This is a very cumbersome procedure. Although capillary tubes were used in the past, it was unacceptable due to problematic procedure and lack of standardization. [22]
The present study aims at modification of the centrifuged buffy coat smear (CBCS) by simplification of the procedure as follows:
1. Use of heparinized micro PCV tubes – this reduces the volume of the blood; it can be obtained even with finger prick.
2. High speed centrifugation – this reduces the centrifugation time.
3. Tubes to be cut with a special instrument in order to avoid shreds of glass pieces.

Method
4. The blood samples were collected in heparinized capillary (haematocrit tubes) and one end of the tube was sealed using bees wax.
5. The tubes were then centrifuged in a micro capillary centrifuge at 6000 rpm for 5 min.
6. The capillary was cut at the junction of buffy coat with plasma and a part of plasma, the buffy coat and a small part of RBC column were pushed onto the slide using a steel wire.
7. Smears were prepared from this material, allowed to air dry, fixed with absolute methanol for 1 min and stained with Field's stain in the same way as PBS was stained. Page 3 of 12 These smears had thickness equivalent to peripheral blood smears, providing an advantage of including the concentration of parasitized RBCs in a thin smear. The smears were examined for a maximum period of 5 min before presenting the negative report.

IV. Results

Out of the 328 patients clinically suspicious of malaria (having fever with chills, nausea or vomiting), 146 patients were positive for malaria. These 146 patients or cases will be referred to as "Study Group" henceforth. Another 200 patients who were not clinically suspicious of malaria will be used to compare the haematological values with study group and will be referred to as "Control Group" henceforth. In our study the maximum numbers of cases were seen in the age group of 20-40yrs (66% of cases). The mean age was 32.4 years. Cases showed a male preponderance with a ratio of 4:1. P. Vivax was the predominant species in our study population comprising 77% of all cases [FIGURE 3&4]. We found that out of 146 cases, 95 cases (65.06%) were positive by PBS, 113 (77.39%) were positive by MCBS and 145 (99.31%) were positive by antigen detection. MCBS detected 12.33% of the cases that were missed on PBS. The antigen detection method though detected 22.61% cases missed on MCBS, however it missed one case of P. vivax that was positive on

PBS and MCBS; hence its sensitivity was calculated to 99.31% (Table 1&2). The analysis of the different methods used is given in the following table. We took antigen as gold standard to compare the other two methods (Table 3). We saw that prevalence of anaemia was higher in females than males by a ratio of 1.5:1 and higher in P. falciparum than P. vivax malaria by a ratio of 2:1. The association of anaemia with malaria was highly significant ($p<0.05$) (Table 4). Prevalence of thrombocytopenia was more in malaria cases than controls by a ratio of 3.4:1. Also prevalence was more in P. falciparum than P. vivax by a ratio of 1.05:1. Association of thrombocytopenia with malaria was highly significant ($p<0.05$) (Table 5). Both cases and controls showed normal counts in 63.69% and 71.5% cases respectively. However, we found that there was a significant association between leucocytosis and malaria ($p<0.05$) and leukopenia and malaria ($p<0.05$). There was no significant difference in Leucocyte counts among malaria species. There was no statistically significant association between malaria and monocytosis, neutropenia, lymphocytosis, lymphocytopenia, basophilia and raised RDW. ($p>0.05$). However, there was significant association between neutrophilia and eosinophilia and malaria ($p<0.05$) in our study. Sensitivity and specificity as well as mean values of haematological parameters is mentioned in Table 6&7.

V. Discussion

Microscopic examination remains the "gold standard" for laboratory confirmation of malaria [3]. It is an established, relatively simple technique that is familiar to most laboratorians. It can confirm the presence of parasites in patient's blood, determine the species and also give an idea of the degree of parasitemia. The smears are able to provide all 3 of these vital pieces of information to the doctor to guide the initial treatment decisions that need to be made acutely [4]. However, microscopic parasitological diagnosis requires continued personnel training and supervision in addition to a minimum laboratory structure. Additionally, such a test is prone to large observer-related variation and lacks sensitivity when performed by non-expert laboratory microscopists [5]. In the present study out of 146 cases, 95 (65.06%) cases were diagnosed on smear out of which 76 (68.85%) were P. vivax and 17(54.54%) were P. Falciparum. 1 case was diagnosed as having mixed infections (Table 8). MCBS could detect 77.39% cases in our study (Table 9). Our study showed that MCBS performed through capillary tubes was easy to perform, affordable and could detect 12.33% cases more than PBS. However, it took more time for preparation and examining as compared to PBS. Table 10 shows comparison of MCBS with other techniques. The antigen detection kit we used had Pan Specific Aldolase (P. vivax and P. falciparum) and P. falciparum specific Lactate Dehydrogenase (pLDH). In our study, none of the cases of P. falciparum was missed by pLDH detection but one case of mixed infection was given as positive for P. falciparum. Aldolase missed one case of P. vivax which was positive on PBS and MCBS. This might have been due to low level antigenemia in early infection. Aldolase also detected two control cases as positive for vivax malaria. They were followed up and were found to be treated cases. They might have been positive due to persistent antigenemia. PLDH and aldolase sensitivity and comparison with other methods is shown in Table 11 and 12. Page 4 of 12 Thrombocytopenia was present in 78.76% of the patients in our study. There was no significant difference in thrombocytopenia between P. vivax cases (78.21%) and P. falciparum cases (81.81%). There was a strong association between thrombocytopenia and malaria ($p<0.05$). No definitive mechanism has been described for thrombocytopenia but decreased megakaryopoeisis can be excluded because platelet forming megakaryocytes are usually normal or increased in the bone marrow [5]. Immune mediated destruction of circulating platelets has been postulated, and it has been found that malaria patients have elevated levels of platelet bound IgG [5][6][7]. Another proposed mechanism is that of platelets engulfing malarial parasites and in the process becoming damaged and thus being removed from circulation [5]. Anaemia was seen in 57.33% cases (P. vivax 46.42%, P. falciparum 75.75%). The association of anaemia with malaria was statistically significant ($p<0.05$). The pathogenesis of anaemia in malaria is multifactorial. A complex chain of pathogenic processes involving mechanical destruction of parasitized RBCs, marrow suppression, ineffective erythropoiesis and accelerated immune destruction of non-parasitized RBCs has been implicated [8].

Our study showed leucocytosis, leukopenia, neutrophilia and eosinophilia to be significantly associated with malaria. The findings of our study were concordant with other studies (Table 13-21).

VI. Conclusion

Microscopic examination remains the "gold standard" for laboratory confirmation of malaria [3]. It is an established, relatively simple technique that is familiar to most laboratorians. It can confirm the presence of parasites in patient's blood, determine the species and also give an idea of the degree of parasitemia. The smears are able to provide all 3 of these vital pieces of information to the doctor to guide the initial treatment decisions that need to be made acutely [4]. However, microscopic parasitological diagnosis requires continued personnel training and supervision in addition to a minimum laboratory structure. Additionally, such a test is prone to large observer-related variation and lacks sensitivity when performed by non-expert laboratory microscopists [5]. In

the present study out of 146 cases, 95 (65.06%) cases were diagnosed on smear out of which 76 (68.85%) were P. vivax and 17(54.54%) were P. Falciparum. 1 case was diagnosed as having mixed infections (Table 8). MCBS could detect 77.39% cases in our study (Table 9). Our study showed that MCBS performed through capillary tubes was easy to perform, affordable and could detect 12.33% cases more than PBS. However, it took more time for preparation and examining as compared to PBS. Table 10 shows comparison of MCBS with other techniques. The antigen detection kit we used had Pan Specific Aldolase (P. vivax and P. falciparum) and P. falciparum specific Lactate Dehydrogenase (pLDH). In our study, none of the cases of P. falciparum was missed by pLDH detection but one case of mixed infection was given as positive for P. falciparum. Aldolase missed one case of P. vivax which was positive on PBS and MCBS. This might have been due to low level antigenemia in early infection. Aldolase also detected two control cases as positive for vivax malaria. They were followed up and were found to be treated cases. They might have been positive due to persistent antigenemia. PLDH and aldolase sensitivity and comparison with other methods is shown in Table 11 and 12.

Thrombocytopenia was present in 78.76% of the patients in our study. There was no significant difference in thrombocytopenia between P. vivax cases (78.21%) and P. falciparum cases (81.81%). There was a strong association between thrombocytopenia and malaria ($p<0.05$). No definitive mechanism has been described for thrombocytopenia but decreased megakaryopoeisis can be excluded because platelet forming megakaryocytes are usually normal or increased in the bone marrow [5]. Immune mediated destruction of circulating platelets has been postulated, and it has been found that malaria patients have elevated levels of platelet bound IgG [5][6][7]. Another proposed mechanism is that of platelets engulfing malarial parasites and in the process becoming damaged and thus being removed from circulation [5]. Anaemia was seen in 57.33% cases (P. vivax 46.42%, P. falciparum 75.75%). The association of anaemia with malaria was statistically significant ($p<0.05$). The pathogenesis of anaemia in malaria is multifactorial. A complex chain of pathogenic processes involving mechanical destruction of parasitized RBCs, marrow suppression, ineffective erythropoiesis and accelerated immune destruction of non-parasitized RBCs has been implicated [8].

Our study showed leucocytosis, leukopenia, neutrophilia and eosinophilia to be significantly associated with malaria. The findings of our study were concordant with other studies (Table 13-21).

VII. Conclusion

The Modified Centrifuged Blood Smear method (MCBS) utilizes some special equipment but these are cheap and can be easily mobilized in far flung areas. The technique improves the sensitivity of peripheral blood smear method without compromising on specificity. The principle of centrifugation, which is employed in Quantitative Buffy coat technique, is employed here and direct visualization of parasites is also possible, thus eliminating disadvantages of quantitative buffy coat technique. Out of the haematological parameters assessed in this study, thrombocytopenia and anaemia were found to be statistically associated with malaria. These two parameters present in a clinically suspected case of malaria can point towards the diagnosis of malaria.

Table 1
Results according to methods used for detection in study group

	PV	PF	MIXED	TOTAL POSITIVE	NEGATIVE	TOTAL
PBS	76	18	1	95	233	328
MCBS	90	22	1	113	215	328
ANTIGEN	111	34	0	145	183	328

Table 2
Results according to methods used for detection in control group

	PV	PF	MIXED	TOTAL POSITIVE	NEGATIVE	TOTAL
PBS	0	0	0	0	200	200
MCBS	0	0	0	0	200	200
ANTIGEN	2	0	0	2	198	200

Table 3
Analysis of methods used for detection of malaria

Method	Sensitivity	Specificity	PPV	NPV	kappa coefficient of agreement
PBS	63.33	99.45	98.95	78.44	0.67
MCBS	77.93	99.45	99.12	85.04	0.77

Table 4
Anaemia distribution
(Normal range [21] – males 13-17gm%, females – 12-15 gm %)

	MALES Hb < 13 gm%	MALES with normal Hb (>13 gm%)	FEMALES Hb <12 gm%	Females with normal Hb (12 gm%)	Total
PV	35	54	17	6	146
PF	19	8	6	0	
MIXED	1	0	0	0	
CONTROLS	36	95	27	42	200

Table 5
Distribution of cases according to platelet count
(Normal range [21] 1.5 to 4.1 lakh/ µl)

	COUNT 1-1.5 LAKH/µl	COUNT 50,000 - 1 LAKH/ µl	COUNT <50,000/ µl	COUNT >1.5 LAKH/µl	TOTAL
PV	15	43	29	25	146
PF	4	15	8	6	
MIXED	0	0	1	0	
CONTROLS	1	19	26	154	200

Table 6
Haematological parameters, their sensitivity, specificities and p values

	Sensitivity	Specificity	PPV	NPV	Chi Square	P value
Anemia (Hb<13 males, <12 females)	57.53	68.5	57.14	68.84	23.4	0 (<0.05)
Thrombocytopenia (<1,50,000/mm^3)	78.76	77	71.42	84.61	105.4	0 (<0.05)
Leukocytosis (>10,000/mm^3)	6.84	81.5	21.27	54.51	9.758	0.001 (<0.05)
Leucopenia (<4000/mm^3)	41.74	90	68.25	63.61	21.14	0 (<0.05)
Neutrophilia (>80%)	13.69	96.5	74.7	60.5	12.2	0.0004 (<0.05)
Neutropenia (<40%)	6.16	93	39.13	57.58	0.09	0.75 (>0.05)
Monocytosis (>10%)	08.21	95.5	57.14	58.76	2.04	0.15 (>0.05)
Eosinophilia (>6%)	01.30	72	3.44	50	41.005	0 (<0.05)
Lymphocytosis (>40%)	21.91	81	45.71	58.69	0.445	0.504 (>0.05)
Lymphocytopenia (<20%)	28.76	73.5	44.21	58.56	0.217	0.64 (>0.05)
RDW (>14%)	41.09	59.5	45.71	58.69	0.012	0.911 (>0.05)

Table 7
Haematological parameters Mean values (±2SD)

	Age in years	Hb value (gm%) Mean± 2SD	TLC (/mm3) Mean ± 2SD	Neutrophils (%) Mean± 2SD	Lymphocytes (%) Mean ± 2SD	Eosinophils (%) Mean± 2SD	Monocytes (%) Mean± 2SD	Basophils (%) Mean± 2SD	Platelets (/µl) Mean± 2SD	RDW (%) Mean± 2SD
STUDY GROUP	32.4 (±12.63)	12.32 (±2.50)	5732 (±2509)	64.09 (±14.85)	28.91 (±13.14)	2.34 (±1.25)	4.76 (±3.19)	0.08 (±0.27)	114589 (±79276)	14.16 (±2.49)
CONTROL GROUP	31.57 (±13.09)	13.1 (±2.68)	7919 (±3800)	59.25 (±13.66)	32.48 (±13.1)	4.47 (±2.61)	3.67 (±2.46)	0.37 (±0.51)	217190 (±88431)	13.93 (±1.87)

Table 8
Percentage of cases positive by PBS

Study series	Percentage of positive cases
Singh et al (2010) [9]	51.10%
Batwala et al (2010) [10]	47.2%
Bhandari et al (2008) [1]	86.79%
Binesh Lal Y et al (1999) [11]	97.82%
Present study	65.06%

Table 9
Percentage of cases positive by MCBS

MCBS Method	% Positivity
Bhandari et al (2008) [1]	86.79%
Duangdee et al (2011) [12]	-
Present study	77.39%
Akhtar et al (2010)79 (CBCS)#	93.33%

Table 10
Comparison of MCBS with PBS

STUDY	% cases which were detected by MCBS over and above those detected by PBS (advantage gained through MCBS)
Bhandari et al (2008) [1]	13.21%
Duangdee et al (2011) [12]	27.8%
Present study	12.33%
Akhtar et al (2010) [13] (CBCS)	9.31%

Table 11
Plasmodium lactate dehydrogenase sensitivity and specificity (Pf specific)

PLDH	Sensitivity	Specificity
Palmer et al (2003) [14]	96.8%	99.4%
Ratasimbasoa A. et al (2007) [15]	92.6%	98.1%
Van den Broek et al (2006) [16]	83.6%	97.8%
Moody A (2002) [17]	96%	100%
De Monbrison et al (2004) [18]	84.3%	98.4%
Present study	100%	100%

Table 12
Aldolase sensitivity and specificity (Pan Pv and Pf)

Study series	Sensitivity	Specificity
Farcas G.A. et al (1994) [19]	86.7%	98.7%
De Monbrison et al (2004) [18]	100	98.9%
Miller R.S. (2006) [20]	82.5%	89.8%
Van den Broek et al (2006) [16]	81.4%	89.9%
Present Study	99.1%	99.51%

Table 13
Thrombocytopenia and malaria

Study series	Percentage of malaria cases showing thrombocytopenia.
Abro AH et al (2008) [23]	81% (PV), 87% (PF)
Khan et al (2010) [24]	74%
Memon A. et al (2006) [25]	70%
Bhandary N. et al (1994) [26]	73.89% (PV), 83.3% (PF)
Agravat et al (2010) [27]	81.5%
Maina R et al. (2010) [28]	49%
Lathia TB et al (2004) [2]	60%
Present Study	78.21% (PV), 81.81% (PF)

Table 14
Anemia and malaria

Study series	Percentage of cases showing anemia
Abro AH et al (2008) [23]	63% (PV), 67% (PF)
Khan et al (2010) [24]	61%
Bashawari et al (2002) [29]	59.2%
Jain M et al (2005) [30]	94.28%
Present Study	46.42% (PV), 75.75% (PF)

Table 15
Variation in Total Leucocyte count in malaria

Study series	Normal TLC	Leucocytosis	Leucopenia
Abro AH et al (2008) [23]	84% (PV), 86% (PF)	2% (PV), 4% (PF)	14% (PV), 10% (PF)
Taylor et al (2008) [31]	85%	5.9%	9.2%
Erhart et al (2004) [32]	68.1% (PV), 68.59% (PF)	5.7% (PV), 4.3% (PF)	20.8% (PV), 27% (PF)
Bashawari et al (2002) [29]	78.3%	7.2%	13.3%
Jadhav et al (2003) [33]	81.1%	1.7% (PV), 6.8% (PF)	15.2% (PV), 10.7% (PF)
Present Study	66.07%(PV), 54.54%(PF)	5.35% (PV), 12.12% (PF)	28.57%(PV), 33.33% (PF)

Table 16
Variation in Neutrophil count in malaria

Study series	Normal	Neutrophilia	Neutropenia
Abro AH et al (2008) [23]	93%(PV), 93%(PF)	3% (PV), 3% (PF)	3% (PV), 4% (PF)
Bashawari et al (2002) [29]	67.9%	8.3%	11.6%
Jadhav et al (2003) [33]	79.3%	5.6%	15.1%
Present Study	82.14%(PV), 72.72%(PF)	11.6%(PV), 21.21%(PF)	6.25% (PV), 6.06% (PF)

Table 17
Variation in Monocyte count in malaria

Study series	Normal monocyte count	Monocytosis	Monocytopenia
Abro AH et al (2008) [23]	90% (PV), 90% (PF)	10% (PV), 10% (PF)	3% (PV), 1% (PF)
Bashawari et al (2002)98	72.7%	12.4%	6.1%
Present Study	92% (PV), 90.91% (PF)	8% (PV), 9.09% (PF)	0% (PV), 0%(PF)

Table 18
Variation in lymphocyte count in malaria

Study series	Normal	Lymphocytosis	Lymphopenia
Abro AH et al (2008) [23]	64% (PV), 85% (PF)	0% (PV), 0% (PF)	36% (PV), 15%(PF)
Bashawari et al (2002) [29]	31.2%	13.6%	42.9%
Present Study	45.53% (PV), 45.45% (PF)	24.11% (PV), 33.33% (PF)	30.35% (PV), 27.27%(PF)

Table 19
Variation in Eosinophil count in malaria

Study series	Normal Eosinophil count	Eosinophilia	Eosinopenia
Abro AH et al (2008) [23]	97% (PV), 100% (PF)	0% (PV), 3% (PF)	0%(PV), 0%(PF)
Bashawari et al (2002) [29]	41%	1.8%	57.2%
Present Study	99.11%(PV), 96.97%(PF)	0.8%(PV), 3%(PF)	0%(PV), 0%(PF)

Table 20
Variation in basophil count in malaria

Study series	Normal Basophil count	Basophilia
Abro AH et al (2008) [23]	100%	0%
Bashawari et al (2002) [29]	98.8%	1.2%
Present Study	100%	0%

Table 21
Variation in RDW in malaria

Study series	RDW raised	Normal RDW
Lathia TB et al (2004) [2]	41%	59%
Agravat et al (2010) [27]	95.7%	4.3%
Present Study	41.09%	58.91%

FIGURE 1
Microcapillary centrifuge used in M.C.B.S. technique

FIGURE 2
Microcapillary centrifuge used in M.C.B.S. technique

FIGURE 3
P. Vivax Ring forms on M.C.B.S. (arrow head) (100x) (Field's stain)

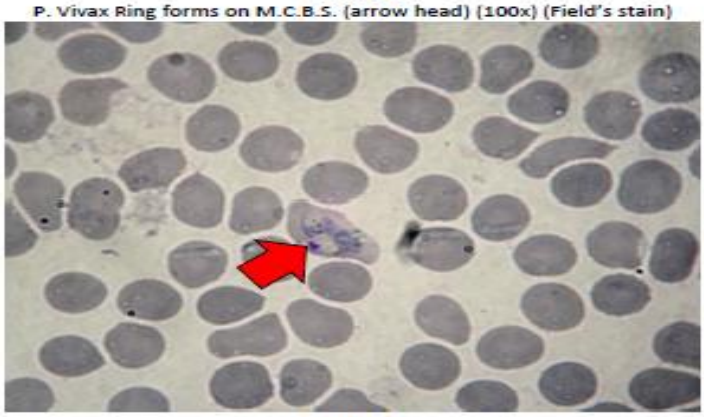
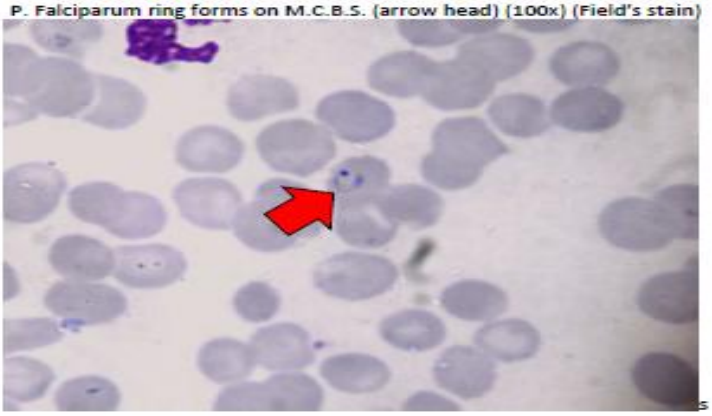


FIGURE 4
P. Falciparum ring forms on M.C.B.S. (arrow head) (100x) (Field's stain)



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