Original Research Article

A comparative study of peripheral blood smear, quantitative buffy coat and antigen detection for diagnosis of malaria

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Abstract

Need of study: Rapid diagnosis is prerequisite for effective treatment and reducing mortality and morbidity of malaria. **Objectives:** To compare and study, the results of Peripheral blood smear, Quantitative Buffy Coat (QBC) and antigen detection for diagnosis of malaria. **Materials and Methods:** A total of 63 samples were collected from patients presenting with classic symptoms of malaria. For traditional microscopy, thick and thin smears were prepared and stained with Leishman's stain, taking it as gold standard. QBC and antigen detection was done using commercially available kits. **Results:** Out of 63 samples, PS study, QBC, Antigen test were positive in 39, 49 and 46 cases respectively. Sensitivity of QBC and malarial antigen test was 100% and 94.8% respectively. **Conclusion:** Peripheral smear study is cost effective, is difficult to interpret for inexperienced microscopist; so if facilities are available, QBC should be used for routine diagnosis. In places where facilities are not available, rapid, simple and easy to interpret antigen detection test can be used.

Key words: Malaria, diagnosis, QBC, antigen detection, Peripheral smear.

Introduction

Malaria is world's most widespread infection. According to the World Malaria Report 2011, malaria is prevalent in 106 countries of the tropical and semitropical world, with 35 countries in central Africa bearing the highest burden of cases and deaths[1]. In 2007, 2.37 billion people were estimated as being at risk of *P. falciparum* malaria worldwide, with 26% located in the WHO AFRO (Africa Regional Office) region compared to 62% in the combined SEARO-WPRO (South-East Asia, Western Pacific Regional Office) regions[2]. Of this total population at risk, about 42% or almost 1 billion people, lived under extremely low malaria risk[2].

Of the five Plasmodia species that infect human beings (*P. falciparum, P. vivax, P. malariae, P. ovale* and *P. knowlesi*), *P. falciparum* and *P. vivax* cause the significant majority of malaria infections. *P. falciparum*, which causes most of the severe cases and deaths, is generally found in tropical regions, such as sub-Saharan Africa and Southeast Asia, as well as in the Western Pacific and in countries sharing the Amazon rainforest. *P. vivax* is common in most of Asia (especially Southeast Asia) and the Eastern Mediterranean, and in most endemic countries of the Americas. In India the most important among the entire vector born diseases is malaria. In 1995, Malaria Action Programme has been launched with emphasis on early diagnosis and prompt treatment, selective and sustainable vector control, out of all these prompt treatment is of first priority.

In the malaria eradication programme case detection through laboratory services is a key element of malaria surveillance[17].

Laboratory confirmation of malaria infections requires the availability of a rapid, sensitive and specific test at an affordable cost.

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Conventional methods of laboratory diagnosis for malaria using microscopic examination of stained thick and thin blood films. However, examination of thick blood films requires technical expertise and availability of good quality microscope.

It is also time consuming and of limited sensitivity in the detection of low parasitemia[18]. Alternative method for malaria diagnosis appropriate for the out patient setting have been introduced to overcome limitations of conventional microscopy. Concentration of malaria parasite infected red blood cell by centrifugation coupled with staining with acridine orange and fluorescence microcopy (quantitative buffy coat)[18].

Most new technology for malaria diagnosis incorporates immuno-chromatographic capture procedure with conjugated monoclonal antibodies providing the indicator of infection. Preferred target antigens are those, which are abundant in all asexual and sexual stages in the parasites. Currently, interest is focused on the detection of Histidine rich protein 2 (HRP2) from plasmodium falciparum and parasite specific lactate dehydrogenase (PLDH) from the parasite glycolytic pathway found in all species[19].

Plasmodium falciparum is most pathogenic of malaria species and is frequently fatal if untreated. Hence, early and rapid diagnosis is required for effective management of patients.

The problem of drug resistance and substitution of newer costlier drugs bring with it the need for rapid, accurate, inexpensive diagnostic procedure.

Aims and objectives

To compare and study, the results of Peripheral blood smear, Quantitative Buffy Coat (QBC) and antigen detection for diagnosis of malaria

Materials and methods

Source of data

63 consecutive patients presented clinically with fever with chills and rigor and other suggestive symptoms of malaria to B.L.D.E.U's Shri. B. M. Patil Medical College Hospital and Research Centre, Bijapur from October 2010 to March 2012.

Sample size

The detection rates of malaria[76] by blood smear is 29%, QBC is 15%, antigen detection test is 14%. The average detection of malaria by all the three method is 19% Considering 95% confidence interval the sample size calculated,

$$n = \frac{(Z_\alpha + Z_\beta)^2 P(1-P)}{d^2}$$

$P = \frac{(29+15+14)}{3} = 19\%$

Z_α= 1.96 for α=0.05

Z_β= 0.842 for β=0.20

d= difference between two method (29-15=14)

n=62

Hence, the minimum of 62 cases will be included in the study to compare blood smear, QBC and antigen detection test for diagnosis of malaria.

Method of collection of data

A detailed physical and systemic examination was performed on all patients presenting with fever chills and rigor and other clinical features suggestive of malaria.

Inclusion criteria

Patients admitted to hospital within the study period, irrespective of age and sex with

1. History of fever with chills and rigors.
2. Fever with or without palpable spleen.
3. Fever with cytopenias.
4. Fever with unconsciousness/hypoglycemia/seizures.
5. Fever with rash.

Exclusion criteria

1. Fever with UTI.
2. Fever with consolidation chest x ray.
3. Fever with sure signs of meningitis.
4. Fever with recently treated with anti-malarials.

All the included patients were subjected to following investigations

- Hb%, TC, DC, ESR. Urine analysis, Random blood sugar, Serum creatinine, Chest X ray
- Peripheral smear for Malaria parasite
- QBC (Quantitative Buffy Coat) for Malaria parasite
- Malarial antigen detection using HRP-2 & p-LDH, Other investigations wherever necessary

Method of test

Sample collection

Oral and written consent was taken from the patients prior to the collection of specimens. Approximately 5ml of venous blood was collected from each patient during the peak of fever and transported to the laboratory.

Preparation of blood smear

1. A drop of blood not larger than a pins head is taken on a grease-free clean slide, at a distance of about half an inch from the right end.
2. Then a spreader is held at an angle of 45 degrees in contact with the drop of blood; then it is lowered lower to an angle of 30 degrees and pushed gently to the left, till the blood is exhausted. As the blood is exhausted, the film begins to form "tails" which should end near about the center of the slide. The spreader may be the smooth edge of a glass slide, with the corners cut off at one end, or a covers lip of a haemocytometer.
3. The film is allowed to dry and labeled by writing across the dried film with a sharp-pointed pencil or a needle.

Characteristics of a Good Thin Film

1. The surface of the film is even and uniform.
2. The margins of the film do not extend to the sides of the slide.
3. The "tail" ends near about the center of the slide
4. It consists of a single layer of red blood cells[54].

Then the smears are stained with Leishman's stain as follows:

1. Leishman's stain is poured from a drop bottle or by means of a pipette over the dried film and is allowed to remain for 30 seconds,
2. The stain is diluted with twice its volume of distilled water, which should be neutral or slightly alkaline (pH 7 – 7.2). It is covered to prevent drying.
3. The diluted stain is allowed to remain on the slide for 10 to 15 minutes.
4. The slide is held under an open tap to flush the stain in a gentle flow of water. The reverse side of the slide is cleaned by rubbing it well with wet and squeezed cotton wool.
5. The dried stained film is examined with 1/12-inch oil-immersion lens.

Note: A properly stained slide has a bluish violet tinge. The correct range of colour is however assured when ionic dissociation of staining radicals occur round about neutral or slightly alkaline pH (7.0 to 7.2). Alkaline buffer solution is particularly necessary to bring out the Schuffner's dots and is prepared as follows: Sodium phosphate 2g, potassium dihydrogen phosphate 1 mg, thymol 1mg and distilled water 1000 ml.

Examination of Thick Blood Film

Preparation

A big drop of blood is taken on a slide and spread with a needle or with the corner of another slide to form an area of a half-inch square; it may also be prepared by taking 4 small drops of blood and joining the corners of the drops with a needle (James, 1920). The thickness of the film should be such as to allow newsprint to be read or the hands of wristwatch to be seen through the dry preparation. The film is dried in a horizontal position and kept covered by a petri dish. It is to be noted that in moist climates, it takes at least half an hour for the thick films to dry at room temperature. Drying may be accelerated by putting the slide inside an incubator.

Staining

It is carried out with Leishman's. The slide should be dehaemoglobinised before staining.

De-haemoglobinization may be carried out in 2 ways:

i. With glacial acetic acid and tartaric acid mixture: The film is flooded with the mixture and as soon as dehaemoglobinisation is complete (indicated by the grayish-white colour of the film), the fluid is drained off by tilting. It is then fixed with methyl alcohol for 3 to 5 minutes. It is then washed thoroughly with neutral or slightly alkaline distilled water so that every trace of acid is removed.

ii. In distilled water by placing the film in a vertical position in a glass cylinder for 5 to 10 minutes. When the film becomes white, it is taken out and allowed to dry in an upright position.

After dehaemoglobinisation, the film is stained with Leishman's in the same way as that used in staining the thin film[55].

QCB Method

The QBC capillary is filled from the AO stained end with the blood sample up to the blue lines, the outer surface is wiped with tissue paper. The capillary is tilted so that the blood flows to the other end, the capillary is tilted for about 10-15 times. The capillary is held horizontally so that the column of blood moves away from the edge of the AO stained end. This end is closed with the finger and the other end is plugged with the plastic closure. The float is inserted inside the capillary using the forceps; the capillary is gently tapped so that the float moves down. The capillary is placed in the QBC centrifuge, which is set at the speed of 12,000 revolutions per minute and spun for 6-8 minutes. The spun capillary is removed and placed in the groove of the capillary holder[57,59].

Observation

The holder is placed on the microscopic stage using transmitted bright light, and the buffy coat is focused under 10x objective. Change the light to epifluorescent and appreciate the red, yellow, green layers. Turn on the transmitted light put a drop of the fluorescent oil on the

capillary, change the objective to 60x and s the granulocyte layer is focused, the holder is moved sideways to appreciate the other layers.
The schizonts and or gametocytes if present will be seen as black-pigmented structures in the lymphocyte layer, without changing the focus it is turned on to the epifluorescent light and transmitted light is switched off. RBC's appear red, yellow/orange–granulocytes, green–lymphocytes, yellow-plasma in that order from the closed end[60].
The malarial parasitic forms are seen as:

1. **Ring forms**

a. Dull green with or without an orange dot at one side, the P.vivax rings are bigger. The P. falciparum rings are smaller, two ring forms may be present very close to each other these are P. falciparum rings the bright green small compact structures are platelets.

2. **P. vivax**

a. Amoeboid forms are seen as dull green irregular structures, the schizonts appear as round dull green and the gametocytes are seen as dull a green big structure, which is confused with the small lymphocytes, which also appear similarly. Under transmitted light if black pigment are seen in these structures it is parasite and if it is not seen they are lymphocytes.

3. **P. falciparum**

a. Gametocytes are seen as green (orange or yellow in old capillary) banana shaped structures having yellowish-green pigments in the center. The plasma layer is also examined ring forms may be seen. If the blood sample is old mosaic of RBC is seen in the background and the parasites may appear orange with yellow pigment. If the capillary is examined after 24 hrs the distinct two colors of the ring forms can be appreciated[59,61].

Antigen detection using HRP-2 and pLDH (malarial antigen detection test)

Procedure

Followed the instruction as by the manufacturer.
Principle of Pf-HRp2:
It utilizes the principle of immuno-chromatography. As the test sample flows through the membrane assembly of the dipstick after placing the clearing buffer, the coloured anti Pf HRP2 antibody colloidal gold conjugate (monoclonal) antibody coated on the membrane leading to formation of pink coloured band, which confirms a positive test result. Absence of this band in the test region indicates a negative test result. The unreacted conjugated and unbound complex if any moves further on the membrane and is subsequently absorbed at the soak pad. Anti rabbit antibodies coated on the membrane at the control region with the rabbit IgG traveling along with the unreacted unbound complex forming a pink band,. This control band serves to validate the test performance[62, 63, 64, 65].

PLDH

It is a good antigenic marker for active malaria infection. A panel of monoclonal antibodies raised against PLDH. The principle is same as that of HRP2[66].

Pan malaria antigen

This is co specific to PLDH for all malarial parasites this has also been utilized in this kit. This also works on the same principle of HRP2[67].

Instructions for use

- Bring the pouch and clearing buffer to room temperature.
- Open the pouch just prior to testing and remove the device
- Collect 5ml of whole blood to be tested using the sample loop or a micropipette.
- Blot the blood on the sample pad in the sample port 'A'
- Dispense four drops of clearing buffer into port 'B'

Positive and Negative for malaria

The PfHRP2 test strips have 2 lines, I for the control and the other for the PfHRP2 antigen. The PfHRP2/PMA test strips and the pLDH test strips have 3 lines, 1 for control, and the other 2 for *P. falciparum* (PfHRP2 or pLDH specific for *P. falciparum)* and non-falciparum antigens (PMA or pan specific pLDH), respectively. Change of color on the control line is necessary to validate the test and its non-appearance, with or without color changes on the test lines, invalidates the test. With color change only on the control line and without color change on the other lines, the test is interpreted as negative.
With the Pf HRP2 test, color change on both the lines is interpreted as a positive test for *P. falciparum* malaria. With the PfHRP2/PMA [The immuno chromatographic test (ICT Malaria P. f. /P.v.test)] and the pLDH tests, color change on the control line and the pan specific line indicates non-falciparum infection and color change on all the 3 lines indicates the presence of *P. falciparum* infection, either as monoinfection or as a mixed infection with nonfalciparum species. Also, if the PfHRP2 line is visible when the PMA line is not, the test is interpreted as positive for *P.falciparum* infection. Mixed infections of *P. falciparum* with the non-falciparum species cannot be differentiated from pure *P. falciparum* infections. However, with regard to the pLDH test, it is claimed that in the presence of *P. vivax* infection, the genus specific line is much darker and more intense than the species specific line due to the presence of all the stages of the parasite in the blood.

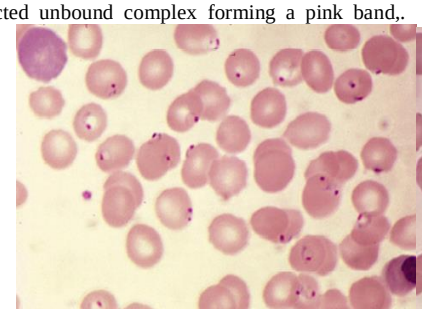

Fig 1: Plasmodium falciparum rings (Lieshman's stain, 1000x)

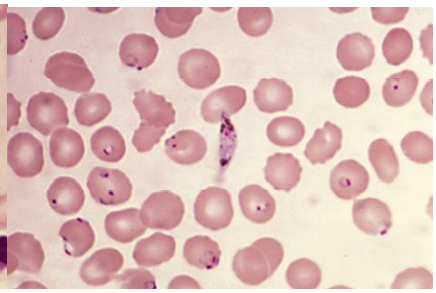

Fig 2: Plasmodium falciparum rings and gamatocytes (Leishman's stain, 1000x)

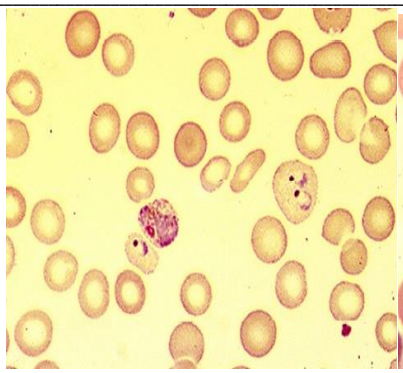

Fig 3: Plasmodium vivax ring and schizont (Leishman's stain, 1000x)

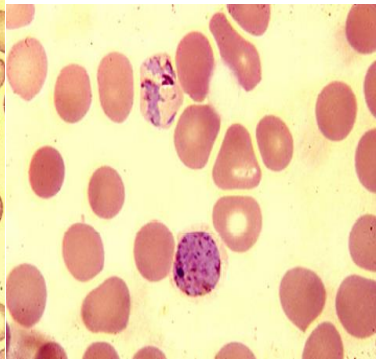

Fig 4: Plasmodium vivax schizonts (Leishman's stain, 1000x)

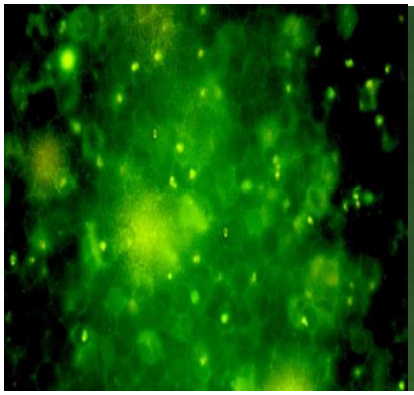

Fig 5: Plasmodium falciparum rings (QBC)

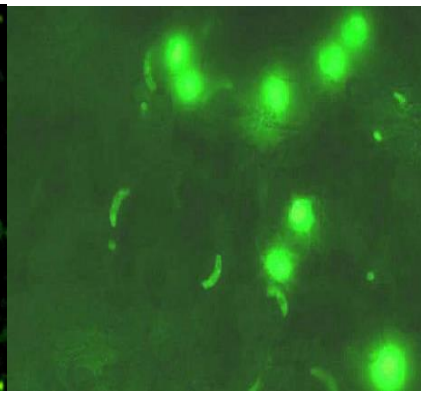

Fig 6: Plasmodium falciparum gamatocytes (QBC)

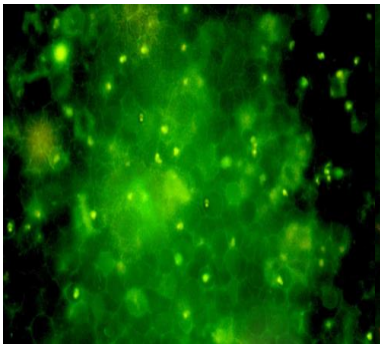

Fig 7: Plasmodium vivax rings (QBC)

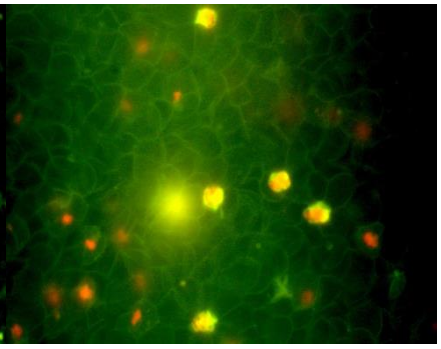

Fig 8: Plasmodium vivax schizonts (QBC)

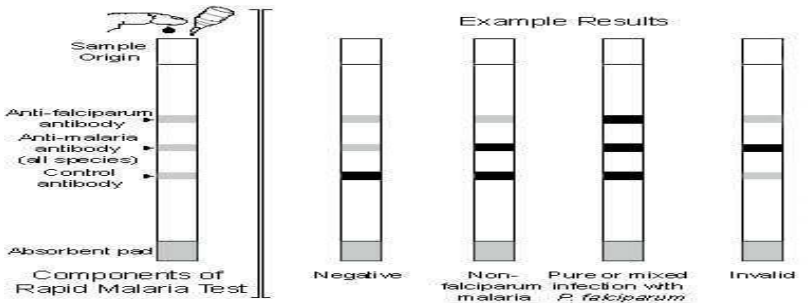

Fig 9: Malarial antigen detection test

Statistical analysis

To analyze the data following methods were applied:

- Mean ± S.D

So to compare the results of three investigations, we applied,

Z (proportion) test

- **Sensitivity**
- **Specificity**
- **Positive predictive values**
- **Negative predictive values** of QBC and Malarial antigen test was compared with Peripheral smear study results.

Results

Study Design

A prospective clinical study consisting of 63 subjects is undertaken to screen the Peripheral smear of patients with clinical diagnosis of malaria and to compare the Rapid Diagnostic test QBC and malarial antigen detection test and to evaluate the diagnostic utility of Rapid Diagnostic test with conventional thick and thin Smear.

Table 1: Age distribution with Sex

Age in years	Sex		Total
	Male	Female	
11-20	4	10	14
21-30	6	5	11
31-40	8	4	12
41-50	7	1	8
51-60	4	5	9
61-70	2	3	5
71-80	1	3	4
Total	32	31	63

In present study, age group ranged from 11 to 80years. More number of cases were seen between 11 to 40 years. Mean age was 39.83 years and standard deviation 18.25 years. Out of 63 patients 32 were male, 31 were female. Male to female ratio is 1.03: 1

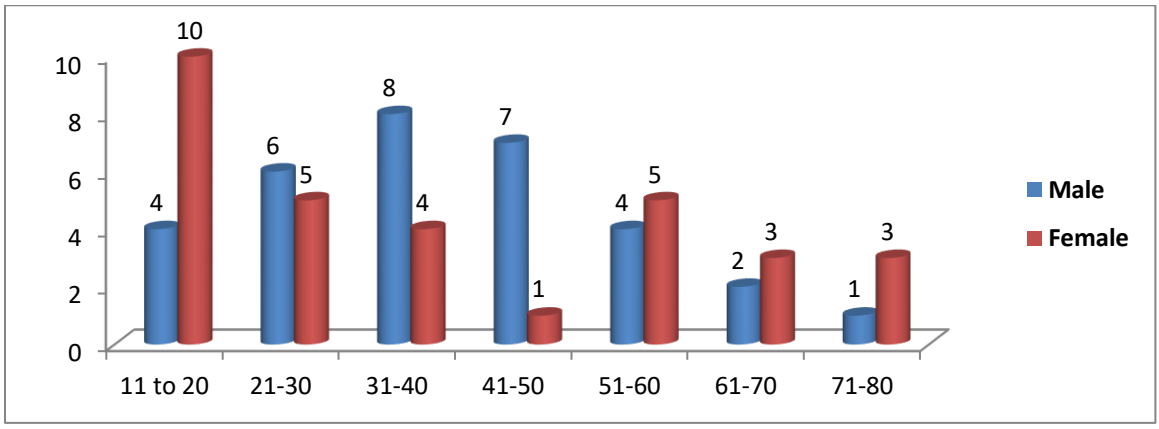

Fig 10: Age distribution

Table 2: Common symptoms with which patients presented

Symptoms	Number of patients	Percentage (%)
Fever	63	100
Head ache	50	79
Nausea and vomiting	13	20
Convulsion	1	0.01
Altered sensorium	1	0.01
Bleeding episodes	0	0
Jaundice & decreased	0	0
Decreased urine output	0	0

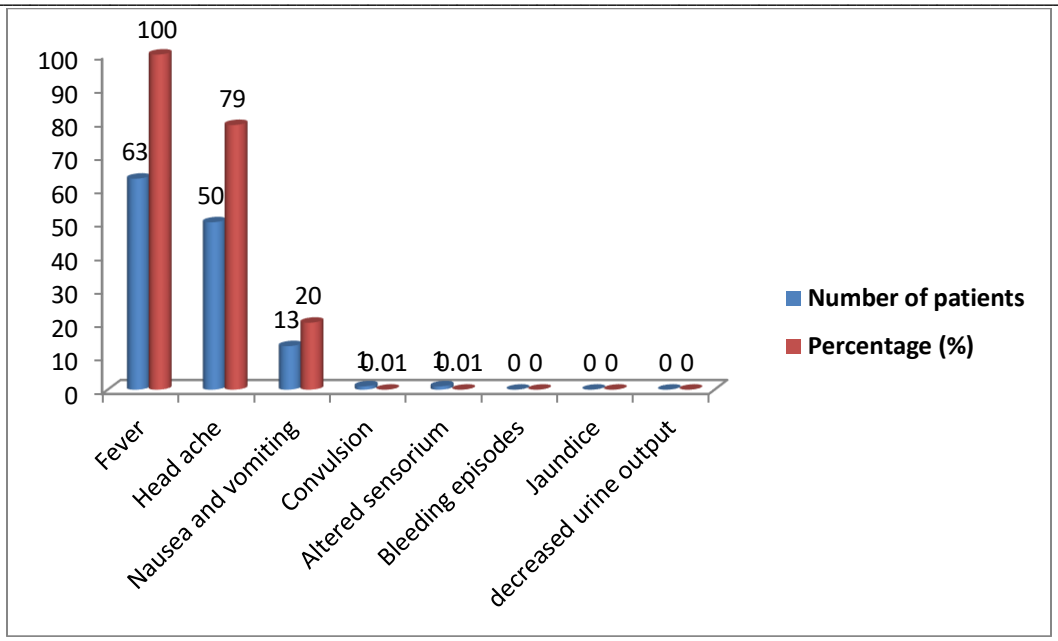

Fig 11: Common symptoms with which patient presented

Fever was present in all the cases (63 patients) studied. The other common presenting symptoms included headache (50 patients), nausea and vomiting (13 patients). Symptoms like convulsions, altered sensorium were encountered rarely.

Table 3: Distribution of cases in relation to Signs

Signs	Number of patients	Percentage (%)
Spleenomegaly	53	84.12
Hepatomegaly	14	22.22
Anemia	23	36.5

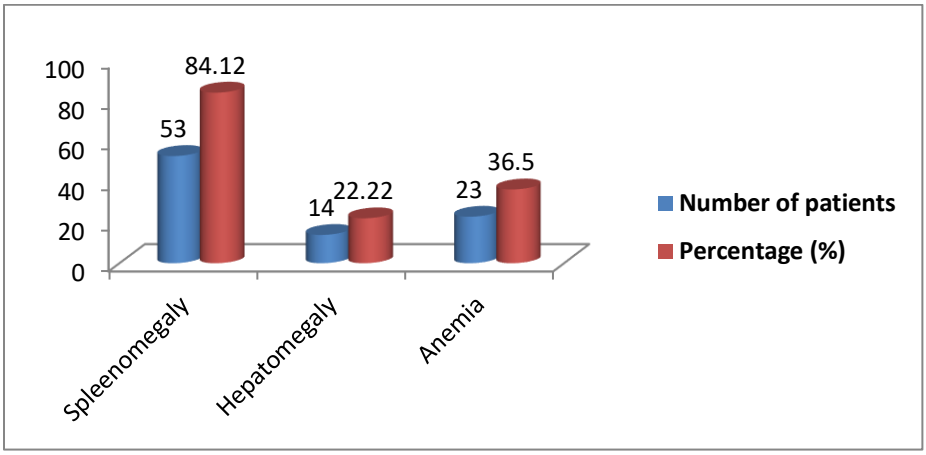

Fig 12: Distribution of cases in relation to Signs

On systemic examination, 53 cases presented with spleenomegaly and 14 with hepatomegaly and 23 patients had anemia. Mean and standard deviation of Hb (haemoglobin) is 10.74 (gram%) and 2.87 (gram%) respectively.

Table 4: Shows the results of Peripheral smear Study.

Peripheral smear study	Number of cases	Percentage (%)
Positive	39	61.9
Negative	24	38.1
Total	63	100

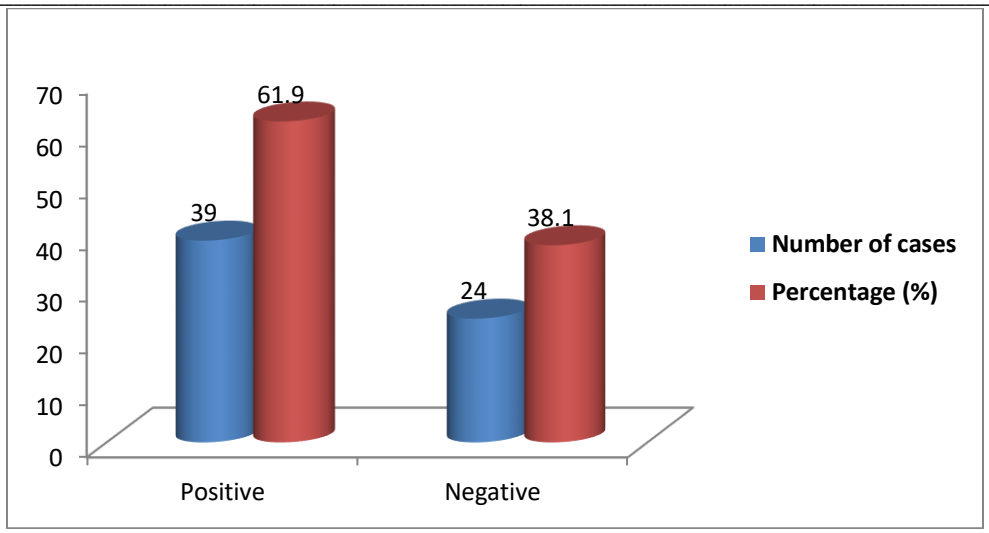

Fig 13: Shows the results of Peripheral smear Study.

Of the 63 cases tested, 61.9% of the cases were positive by Peripheral smear study and 38.1% cases were negative.

Table 5: Shows the distribution of the species of the parasite by Peripheral smear study

Species	Number of cases	Percentage (%)
Plasmodium vivax	27	69.23
Plasmodium falciparum	12	30.77
Total	39	100

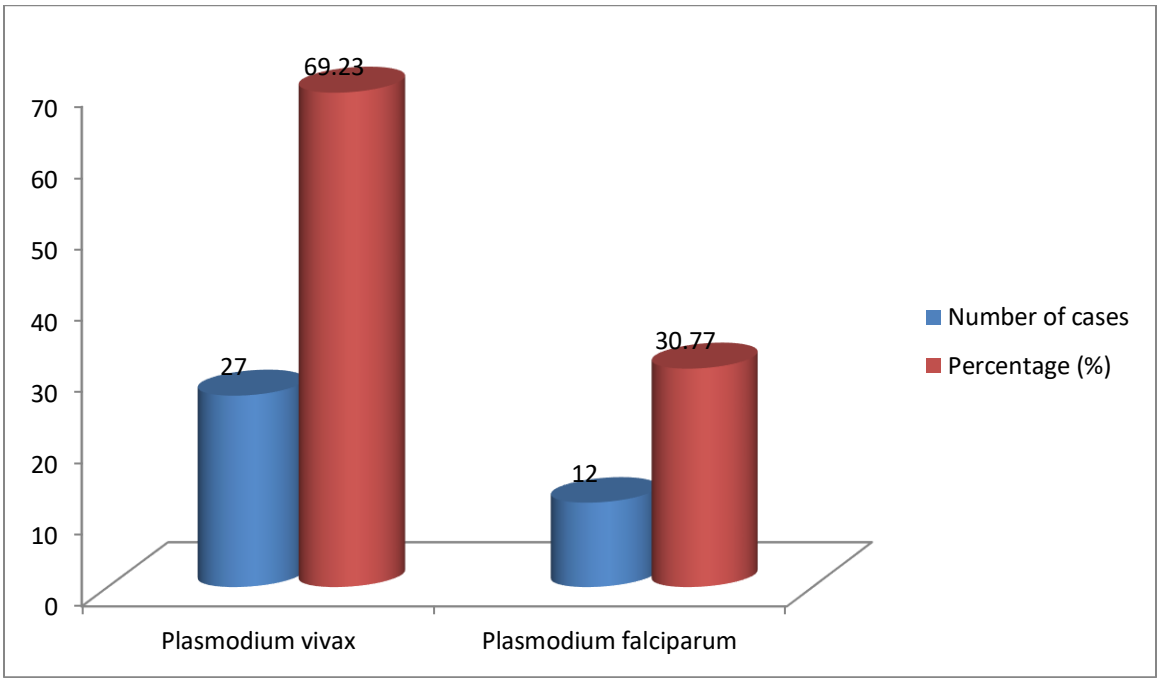

Fig 14: Shows the distribution of the species of the parasite by Peripheral smear study

69.23% of the cases positive by Peripheral smear were that of P. vivax, 30.77% of the cases were of P. falciparum

Table 6: Shows the results of QBC method

QBC	Number of cases	Percentage (%)
Positive	49	77.77
Negative	14	22.23
Total	63	100

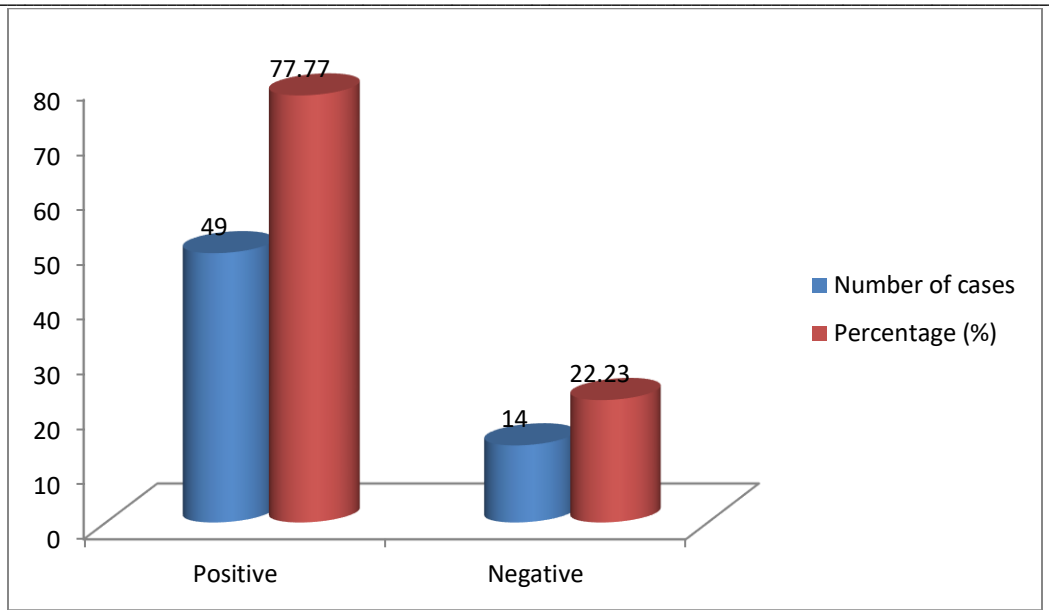

Fig 15: Shows the results of QBC method

Of the 63 cases tested, 49 cases were positive by QBC method and 14 cases were negative.

Table 7: Shows the distribution of the species of the parasite by QBC

Species	Number of cases	Percentage (%)
Plasmodium vivax	27	55.10
Plasmodium falciparum	22	44.90
Total	49	100

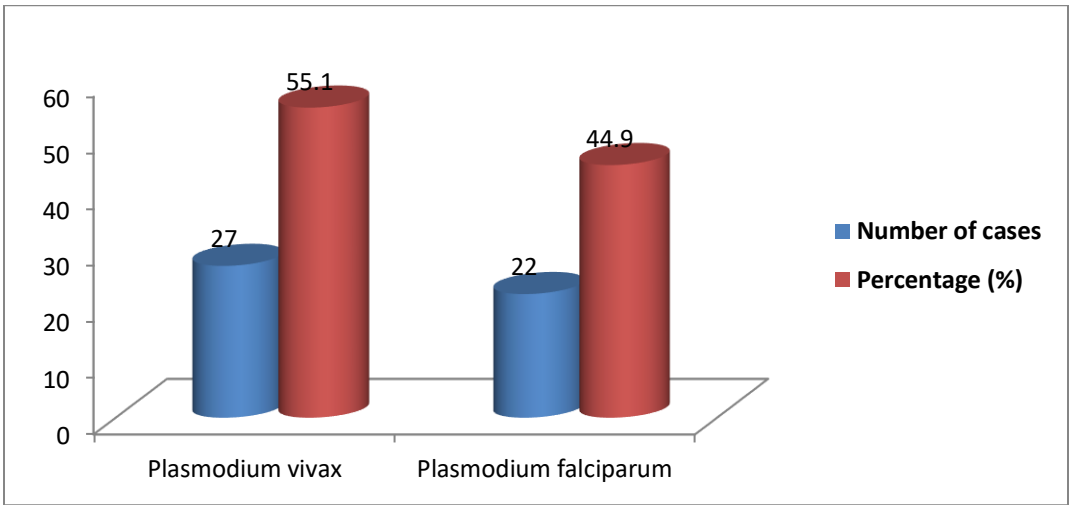

Fig 16: Shows the distribution of the species of the parasite by QBC

55.1% of the 49 cases were positive for Plasmodium vivax and 44.9% of the 49 cases were positive for P. falciparum.

Table 8: Comparison of the results of QBC and Peripheral smear study

QBC	Peripheral smear study		Total
	Present	Absent	
Test positive	39	10	49
Test Negative	0	14	14
Total	39	24	63

14 cases were found to be negative by both the methods. 39 cases were found to be positive by both methods. 10 cases which were negative by Peripheral smear study were found to be positive by QBC.

The QBC method has a sensitivity of 100%, specificity of 58.3%, positive predictive value of 79.5% and negative predictive value of 100% in comparison with Peripheral smear study.

Table 9: Shows the results of Malarial Antigen detection test.

Malarial antigen detection test	Number of cases	Percentage (%)
Positive	46	73.01
Negative	17	26.99
Total	63	100

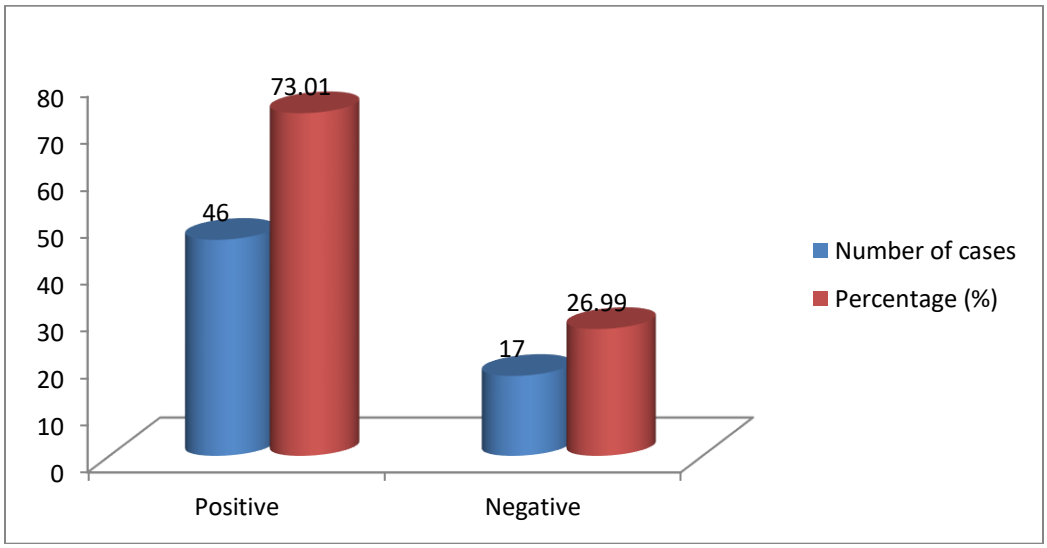

Fig 17: Shows the results of Malarial Antigen detection test

Of the 63 cases tested, 46 cases were positive by Malarial antigen detection test and 17 cases were negative.

Table 10: Shows the distribution of the species of the parasite by Malarial antigen detection test

Species	Number of cases	Percentage (%)
Non falciparum	27	58.69
Plasmodium falciparum	19	41.31
Total	46	100

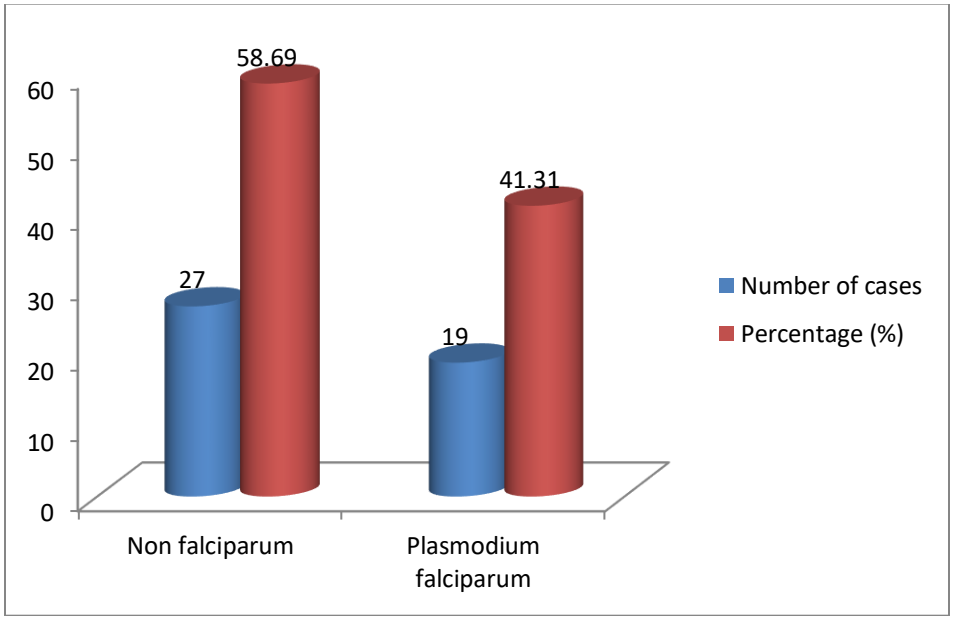

Fig 18: Shows the distribution of the species of the parasite by Malarial antigen detection test

46 cases were positive by Antigen detection using HRP-2 and p-LDH, out of which 19 cases were PF and 27 cases were non-falciparum species (plasmodium vivax, malariae, ovale)

Table 11: Comparison of the results of Malarial antigen detection test and Peripheral smear study

Malarial antigen test	Peripheral smear study		Total
	Present	Absent	
Test positive	37	9	46
Test Negative	2	15	17
Total	39	24	63

15 cases were found to be negative by both the methods. 37 cases were found to be positive by both methods. 9 cases which were negative by Peripheral smear study were found to be positive by Malarial antigen test. this test has detected 9 more cases as positive compared to peripheral smear study, but 2 cases were diagnosed as negative by this test were found positive by peripheral smear study.

Z(proportion)=7.04, P=0.000 (There is a significant difference between Malarial antigen test and peripheral smear study)

The Malarial antigen test has a sensitivity of 94.8%, specificity of 62.5%, positive predictive value of 80.4% and negative predictive value of 88.2% in comparison with Peripheral smear study.

Table 12: Number of cases detected by different methods

Name of the test	No. of cases detected out of 63
Peripheral smear study	39
Quantitative Buffy Coat	49
Malarial Antigen detection test	46

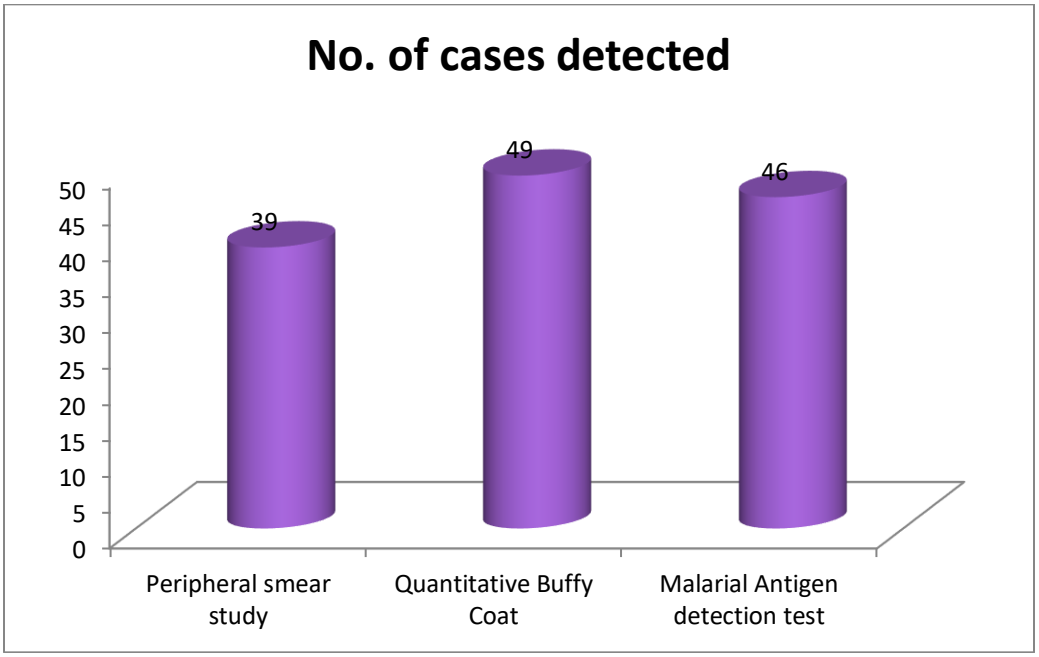

Fig 19: Comparison of Results obtained from different tests

Out of 63 cases, PS study, QBC, Antigen test were positive in 39, 49 and 46 cases respectively.

Table 13: Comparison of QBC and Malarial Antigen detection test with PS study

Name of the test	Sensitivity	Specificity	Positive predictive value	Negative predictive value
QBC	100%	58.3%	79.5%	100%
Malarial antigen detection test	94.8%	62.5%	80.4%	88.2%

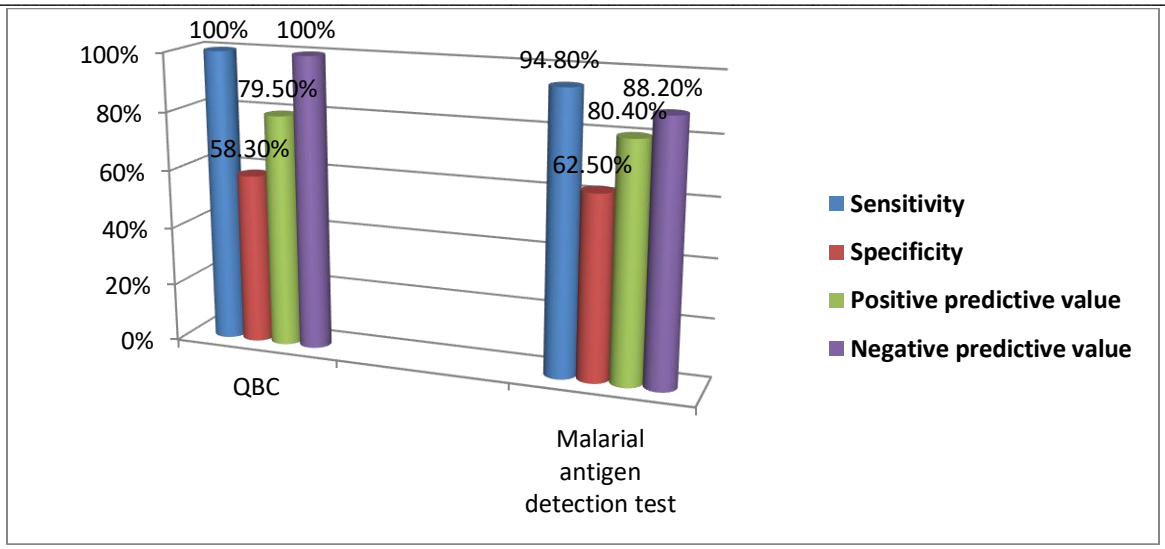

Fig 20: Comparison of QBC and Malarial Antigen detection test with PS study

Sensitivity, Specificity, Positive predictive value, negative predictive value of QBC were 100%, 58.3%, 79.5%, 100% respectively. Sensitivity, Specificity, Positive predictive value, negative predictive value of Malarial antigen test were 94.8%, 62.5%, 80.4%, 88.2% respectively.

Discussion

The results of 63 cases studied are compared with the results of other studies and discussed.

In the present study, mean age of the patients was 39.83 years

Table 14: Mean and Standard Deviation of age compared with other studies

Study series	Mean	S.D. (Years)
Rickman et al (1989)	30.6	15.9
Kodsinghe et al(1997)	26	15.8
Mills et al (1999)	39	-
Present study	**39.83**	**18.25**

Table 15: Male to Female ratio compared with other studies

Study series	Ratio
Ugen et al (1995)	1.3:1
Kodsinghe et al (1997)	2:1
Mishra et al (1999)	3:1
Singh et al (2001)	1.9:1
Present study	**1.03:1**

Male to female ratio is 1.03:1, i.e., men and female are almost equal in number.

Peripheral smear

Peripheral smear is the standard, cost effective diagnostic technique for detection and differentiation of plasmodium species.

It has several limitations like, time consuming, labour intensive and requires the service of skilled technician. Further diagnosis of malaria can be missed if the parasite count is less than 60/µL of blood.

Of the 63 cases tested, 61.9% of the cases were positive by Peripheral smear study which is comparable with the study of Rickman et al (55.5%)[68]. Rest of the 10 cases were not diagnosed due to low parasite count.

The percentage of positivity by peripheral smear compared with other studies.

Parasite species

In the present study, the number of cases of P. falciparum and P. vivax were 12 and 27 respectively as per peripheral smear study only. But taking into consideration positivity by any of the 3 methods studied the corresponding figures for P. falciparum and non-falciparum (P. vivax, ovale, malariae) were 22 and 27 respectively. These additional cases would have been missed if only Peripheral smear study was done.

Quantitative Buffy Coat

It is a highly sensitive and specific diagnostic technique. It has the advantage of rapid, easy interpretation and the cases can be diagnosed inspite of low parasitemia.

It is more sensitive in detecting P. falciparum gametocytes, P. vivax schizont and less sensitive in detecting ring stages of P. vivax and P. falciparum and cases of mixed infection. The only draw back is its cost factor. In the present study 49 cases were diagnosed out of which 27 cases (55.10%) were P. vivax and 22 (44.9%) were P. falciparum.

The overall sensitivity of QBC method in the present study was high. Specificity was difficult to interpret. Inability to attain a higher level could be partly explained by the fact that a Peripheral smear study of only 100 microscopic fields was done. A rigorous search for parasites by reading more than 100 fields was not done. The intent was to mimic as much as possible actual blood film reading criteria practiced at the malaria clinics while obtaining results that were reliable and not affected by work load and time constraints encountered.

The number of cases detected by QBC method were more. Ten cases with low density parasitaemia that were missed by Peripheral smear

were apparently picked up by QBC method. Nine cases which were also positive by Malarial antigen detection test. It was evident that QBC was capable of detecting more malaria cases than Peripheral smear in this study.

Table 16: Sensitivity and specificity of Malarial antigen detection test compared with other studies

Study series	Sensitivity (%)	Specificity (%)
Beadle et al (1994)[45]	39-100	87-98
Craig et al (1997)[48]	96.67	>95
Singh et al (1997)[75]	93	92
Mishra et al (1999)[61]	97	100
Huong NM et al (2002)[77]	95%	97.2%
Anthony Moody (2002)[72]	100%	100%
Parija et al (2009)[76]	75	100
Present study (2011)	**94.8**	**64.5**

The Malraial antigen detection test will be particular use in rapid diagnosis of febrile patients. Furthermore, because inexperienced microscopists often have difficulty in detecting less than 60 parasites/ µL, the Malarial antigen detection test has greater sensitivity than Peripheral blood smear.

Peripheral smear

Microscopic analysis of appropriately stained thick and thin blood smears has been the standard diagnostic technique for identifying malaria infections for more than a century. The technique allows for detection and differentiation of the Plasmodium species. Standardisation of the method makes it possible to obtain an estimation of the number of circulating parasites per µL. A limited amount of equipment is required, mainly a microscope and facilities for preparing stained blood films. The technique is capable of accurate and reliable diagnosis when performed by skilled microscopists using defined protocols.

QBC method

It was observed that QBC method was highly sensitive and specific even in specimen with low density parasitaemias(2 parasites/ul)[79]. The major advantages of the QBC tube over the Peripheral smear study are its speed and ease interpretation. Application of QBC in the field has several advantages which include overall sensitivity and ability to detect P. falciparum gametocytes and P. vivx schizonts more frequently than Peripheral smear.

Conclusion

Since malaria is endemic in certain regions of India, we need to employ more sensitive test, which are also rapid to detect low levels of parasitemia in population. Therefore, we recommend QBC to be used in setups where appropriate facilities are available: in situations where adequate laboratory back up is not available, simpler and easy to use techniques like antigen detection can be employed, however peripheral smaear study still remain the gold standard for identification of species.

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