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# Clinical Profile Of Malaria Cases Detected By Peripheral Smear And Evaluation Of Diagnostic Tests In Malaria.

**U Sasireka, K Kamatchi, and K Santhi*.**

Assistant Professor, Department Of Pediatrics, Government Karur Medical College, Karur, Tamil Nadu, India.

**ABSTRACT**

Malaria is a major global infectious disease caused by parasitic protozoans of the genus Plasmodium. Infections in humans primarily involve four Plasmodium species: Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae,. In the 20th century, malaria was a dreaded disease. With the efforts of public health agencies and the availability of artemisinin derivatives, morbidity and mortality associated with malaria have decreased and there is a revival of hopes that malaria will be eradicated from our country in the coming decades. Despite recent reductions in the overall malaria case incidence, malaria remains an important cause of morbidity and mortality. Clinical profile of malaria cases detected by peripheral smear and evaluating the diagnostic value of Quantitative Buffy Coat(QBC) and Rapid Diagnostic Test (RDT using pLDH ) against the gold standard peripheral smear examination, in the diagnosis of malaria in children between 1month to 12 years of age group. This descriptive study and the case-control study were conducted in 2019 at the department of Pediatrics, government Karur medical college, Karur. 150 children were enrolled in the study. The sample size was calculated based on observations from previous studies, as having a sensitivity of 88% with 10% allowable error and 99% confidence.150 children satisfying the case definition and inclusion criteria were selected and subjected to peripheral smear, QBC & RDT(pLDH). The smears were obtained as per the standard technique and stained with Leishman's stain. A minimum of 200 oil immersion fields (x 100 objectives) were examined in the thick film. Following the detection of malarial parasites in a thick film, the thin film was examined to determine the species. If the malarial parasites were absent in the thick smear, the entire thin film was examined. Anemia was detected among 44 cases of smear positive malaria. The possibility of a child having anemia is 9.2 times more common among smear positive cases when compared to smear-negative cases OR(95% confidence interval)=9.2(4.3-19.6). Thrombocytopenia was observed in 38(57.5%) malaria cases with an odds ratio of 55.65 and 95% confidence interval of 12.6-245 which was statistically very much significant. The other investigations (continuous variables) like total count, blood sugar and urea, serum creatinine, and bilirubin were not statistically significant when analyzed with a two-sample t-test between smear positive and smear-negative cases. The RDT (pLDH) test showed 82 cases were positive for malaria. Among the 82 cases, 74 (90.2%) were positive for *P. vivax* and 8 were positive for *P. falciparum* ( 9.8%). A case that was negative by RDT (pLDH) test was found to be positive by smear as well as QBC. The technique and interpretation of RDT (pLDH) is much easier when compared to peripheral smear and QBC. RDT (pLDH) can be useful in areas where specialized laboratories or microscopy are unavailable and when an urgent malaria diagnosis is needed by a practitioner without the delay associated with the laboratory diagnosis.

**Keywords**: Malaria, Peripheral smear study, QBC, RDT(pLDH) test

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***Corresponding author***

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## INTRODUCTION

Malaria, called the King of diseases is caused by plasmodium infection. It's the most important infectious disease in tropical and subtropical regions and continues to be a major public health problem. Over 40% of the world's population is exposed to the risk of malaria. There are four species of plasmodium that cause malaria: *P. vivax*, *P. malariae, P. ovale, P. falciparum. P. vivax* infection is the most common. Infection by *P. falciparum* is the most serious, being responsible for deaths among children [1]. The sporozoite is transmitted to the host by an anopheles mosquito. Transmission may also occur trans placentally and rarely through blood. The magnitude of the problem is further enhanced by *P. falciparum* resistance to standard antimalarial drugs, adding to increased mortality and morbidity [2]. Hence our efforts should be directed towards more restrictive use of the drugs and uniform prescribing practices to limit the spread and intensification of drug resistance. A remarkable decrease in antimalarial drug use could be achieved by improving the diagnosis of malaria [3]. Though, microscopy is considered the gold standard method for diagnosing malaria, it is time-consuming, labor- intensive, and requires considerable expertise for its interpretation, and variable sensitivity and specificity compared to the recent technical advances, particularly at low levels of parasitemia. The majority of malaria cases contributed by the developing countries, where cost-effectiveness is very much is important [4]. The urgency of obtaining results with suspected acute malaria makes some of the sensitive methods for diagnosis of malaria highly difficult. Availability of a rapid, simple, and accurate test could greatly aid in the early diagnosis of malaria including in remote areas, where health facility coverage is low [5].

The classic presentation of malaria consists of paroxysms of fever alternating with periods of fatigue but otherwise relative wellness. Febrile paroxysms are characterized by high fever, rigors, sweats, and headache, as well as myalgia, back pain, abdominal pain, nausea, vomiting, diarrhea, jaundice, splenomegaly, hepatomegaly, anemia, thrombocytopenia, a normal or low leukocyte count, or any combination of these manifestations. Paroxysms coincide with the rupture of schizonts that occurs every 48 hr with P. vivax and *P. ovale* and result every other day fever spikes [6]. Rupture of schizonts occurs every 72 hr with *P. malariae* and results in fever spikes every 3rd or 4th day. Periodicity is less apparent with P. falciparum and mixed infections. Severe, high-risk malaria is characterized by a depressed level of consciousness, seizures, irregular respirations or airway obstruction, hypoxia, hypotension, tachycardia, dehydration, hypoglycemia, metabolic acidosis, and hyperkalemia [7]. A clinical diagnosis of malaria is still challenging because of the vague signs and symptoms, which overlap with other common infections. The overlapping of malaria symptoms with other tropical diseases impairs diagnostic specificity, which will lead to the indiscriminate use of antimalarials and compromise the care for patients with non-malarial fevers. Peripheral smear( Thick and thin smear ) microscopy is considered to be the gold standard test for diagnosing malaria [8].

## METHODS

This Descriptive study and the case-control study were conducted in 2019 at the department of Pediatrics, government Karur medical college, Karur. 150 children were enrolled in the study. The sample size was calculated based on observations from previous studies, as having a sensitivity of 88% with 10% allowable error and 99% confidence.150 children satisfying the case definition and inclusion criteria were selected and subjected to peripheral smear, QBC & RDT (pLDH).

**Inclusion criteria**: Children between 1 month to 12 years of age group presented with fever for more than 5 days with other clinical symptoms and signs of malaria-like fever with chills and rigor, pallor, hepatosplenomegaly, etc. Pallor: palmar pallor is taken into account as per IMNCI guidelines. Hepatomegaly: If the liver span for that particular age is more than normal, it was taken as hepatomegaly. Splenomegaly: palpable spleen isconsidered splenomegaly.

**Exclusion criteria**: Fever with the obvious focus of infection like an abscess, urinary tract infection (which will cause fever with chills), and children whohad received treatment for malaria in the past 4 weeks.

The peripheral smears were obtained as per the standard technique and stained with Leishman's stain. A minimum of 200 oil immersion fields (x 100 objectives) were examined in the thick film. Following

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the detection of malarial parasites in a thick film, the thin film was examined to determine the species. If the malarial parasites were absent in the thick smear, the entire thin film was examined.

For the QBC technique, approximately 60 µl of blood was taken into a capillary tube which is coated with acridine orange from a blue-lined end and fitted with a cap. Then a plastic float was inserted inside the capillary tube and centrifugation was done. The tube was then mounted on a small plastic holder and examined by rotating the tube under an ordinary light microscope with customized fluorescence.

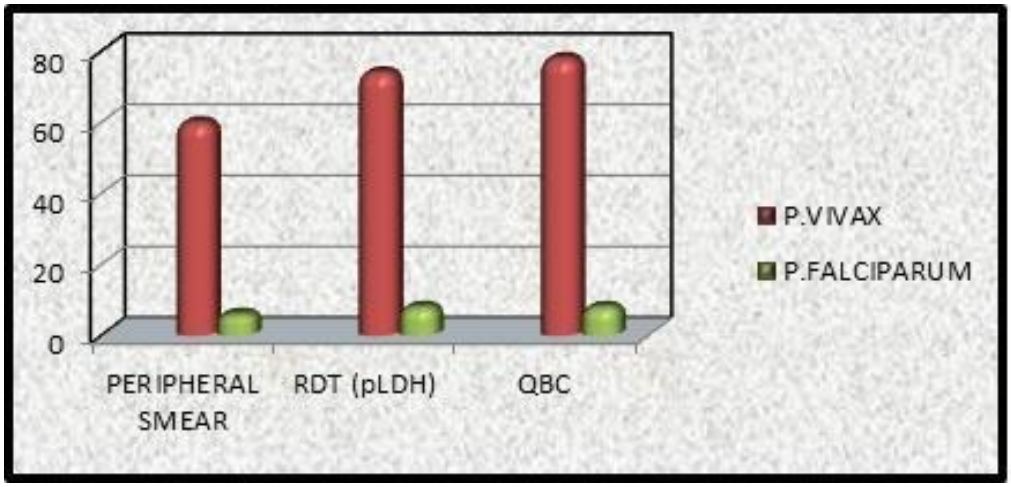
Plasmodium lactate dehydrogenase immunochromatographic assay was done using the commercial kit DiaMed OptiMal-IT flow, inc., The device was placed horizontally on a flat surface and the patient's name and IP number were written on the label. One drop of buffer to the first (conjugate) well, and four drops to the second (wash) well were added and wait for 1 minute. Blood was taken up to the black mark on the pipette. An entire volume of blood (10µml) was added to the first well. The mixture was gently stirred with the upper end of the pipette and allowed to stand for 1 minute, during which the lysis buffer disrupts the RBCs to release pLDH. The dipstick holder was pulled out, the legs of the dipstick holder were inserted into the holes beside the conjugate well so that the dipstick end reaches the bottom of the conjugate well. For the next 8 min, the blood/conjugate mixture is allowed to migrate to the top of the pLDH strip. The dipstick is transferred to the second well with the washing buffer which clears the hemoglobin from the strip. Once the reaction field is completely cleared of blood, and the control band is visible, the dipstick was removed from the wash well and fixed back into the clear plastic frame & observed for the presence of any band and the corresponding letter C, P & Pf.

**Statistical analysis:**

Data was entered into a Microsoft office excel sheet and statistical operations were done through SPSS for windows version 17. All the univariate analyses were done by chi-square test. For continuous measurements, a two-sample t- test was done. sensitivity, specificity, positive predictive value, negative predictive value, positive and negative likelihood ratios, and diagnostic accuracy were calculated for QBC and RDT (pLDH) by comparing the results with the gold standard peripheral smear study.

## RESULTS

**Graph1: Smear Positive Cases**




Graph :1 66. *P. vivax*- 60 cases, *P. falciparum*- 6 cases. RDT (pLDH) positive cases: 82. *P. vivax*-74 cases, *P. falciparum*-8 cases. QBC positive cases: 86. *P. vivax*-78 cases, *P. falciparum*-8 cases

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**Table 1: Correlation of clinical features and investigations with peripheral smear results univariate analysis**

| Variables | | Peripheral smear positive | Peripheral smear negative | OR | 95% C.I | P value |
|---|---|---|---|---|---|---|
| Myalgia | Present | 10(15.2%) | 23.8% | 0.188 | 0.2,1.3 | 0.571 |
| | Absent | 56(84.8%) | 76.2% | | | |
| Chills | Present | 13(19.7%) | 26.2% | 0.351 | 0.3,1.5 | 0.691 |
| | Absent | 53(80.3%) | 73.8% | | | |
| GIT symptoms | Present | 27(40.9%) | 61.9% | 0.426 | 0.2,0.8 | 0.11 |
| | Absent | 39(59.1%) | 38.1% | | | |
| Headache | Present | 11(16.7%) | 8.3% | 2.2 | 0.8,6 | 0.12 |
| | Absent | 55(83.3%) | 91.7% | | | |
| Seizure | Present | 3(8.3%) | 8.3% | 0.524 | 0.1,2.1 | 0.36 |
| | Absent | 63(91.7%) | 91.7% | | | |
| Altered sensorium | Present | O(0%) | 4.8% | 0.548 | 0.,0.6 | 0.07 |
| | Absent | 66(100%) | 95.2% | | | |
| Renal symptoms | Present | 1(1.5%) | 1.5% | 0.415 | 0.04,4 | 0.44 |
| | Absent | 65(98.5%) | 98.5% | | | |
| Pallor | Present | 36(54.5%) | 40.5% | 1.765 | 0.9,3.3 | 0.09 |
| | Absent | 30(45.5%) | 59.5% | | | |
| Icterus | Present | 3(4.5%) | 0% | 0.429 | 0.3,0.5 | 0.05 |
| | Absent | 63(95.5%) | 100% | | | |
| Edema | Present | 0(0%) | 2.4% | 0.558 | 0.48,0.6 | 0.21 |
| | Absent | 66(100%) | 97.6% | | | |
| Hepatomegaly | Present | 8(12.1%) | 15.5% | 0.75 | 0.29,1.9 | 0.56 |
| | Absent | 58(87.9%) | 84.5% | | | |
| Splenomegaly | Present | 14(21.2%) | 17.9% | 1.238 | 0.5,2.7 | 0.60 |
| | Absent | 52(78.8%) | 82.15 | | | |
| Hepato Splenomegaly | Present | 36(54.55%) | 41.7% | 1.680 | 0.87,3.2 | 0.12 |
| | Absent | 30(45.5%) | 58.3% | | | |
| Anemia | Present | 44(66.6%) | 17.8% | 9.2 | 4.3,19.6 | 0.000 |
| | Absent | 22(33.4%) | 82.2% | | | |
| Thrombo - cytopenia | Present | 38(57.5%) | 2% | 55.64 | 12.6,245 | 0.000 |
| | Absent | 28(42.5%) | 98% | | | |

From table 1, we can infer that no individual clinical parameter is statistically significant with peripheral smear results, which necessitates the need for diagnostic tests for the diagnosis of malaria. Anemia was detected among 44 cases of smear positive malaria. The possibility of a child having anemia is 9.2 times more common among smear positive cases when compared to smear-negative cases OR(95% confidence interval)=9.2(4.3- 19.6). Thrombocytopenia was observed in 38(57.5%) malaria cases with an odds ratio of 55.65 and 95% confidence interval of 12.6-245 which was statistically very much significant. The other investigations (continuous variables) like total count, blood sugar and urea, serum creatinine, and bilirubin were not statistically significant when analyzed with a two-sample t-test between smear positive and smear-negative cases.

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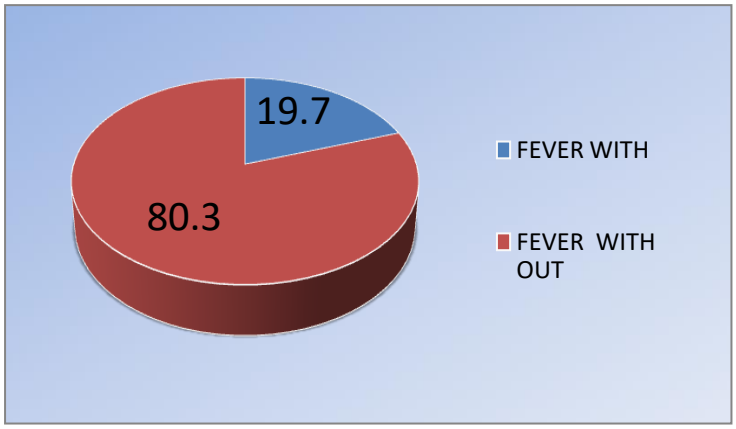
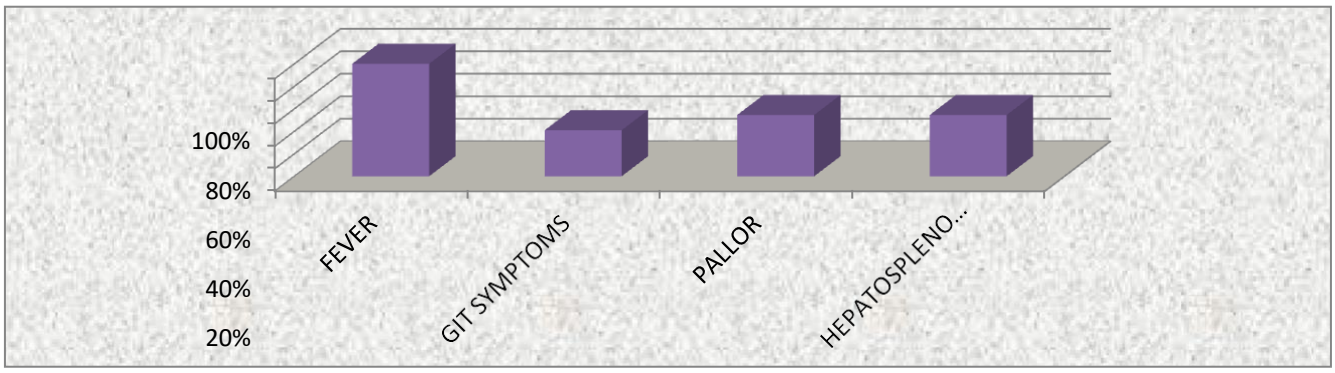
**Graph 2: Fever and chills**



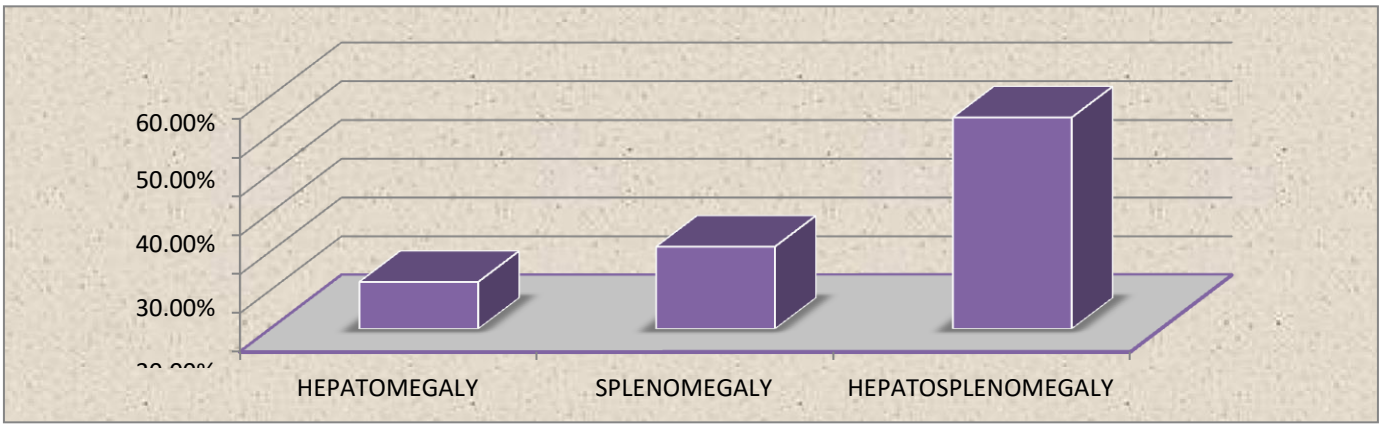

Graph 2: Total smear positive cases: 66, The mean for fever duration was 8.5 days (SD 5.07) Fever withoutchills: 53 (80.3%) Fever with chills: 13 (19.7%)

**Graph 3: Major clinical features of malaria**




Graph 3: Fever 100%, GIT symptoms:27(40.9%),Pallor:36(54.5%), Hepatosplenomegaly:36(54.5%)

**Graph 4: Organomegaly in malaria**




Graph 4: Hepatomegaly: 8(15.5%), Splenomegaly: 14(21.2%), Hepatosplenomegaly: 36(54.5%)

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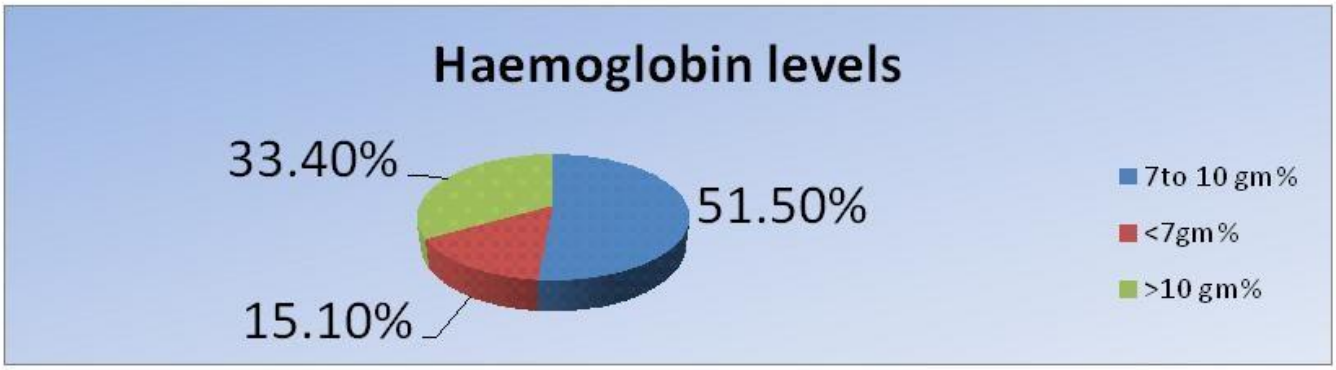
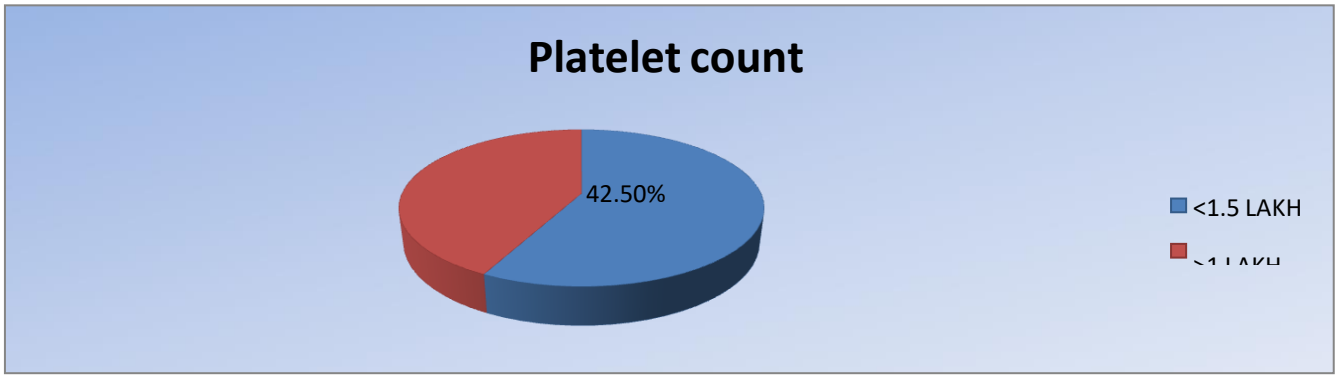
**Graph5: Haemoglobin levels**



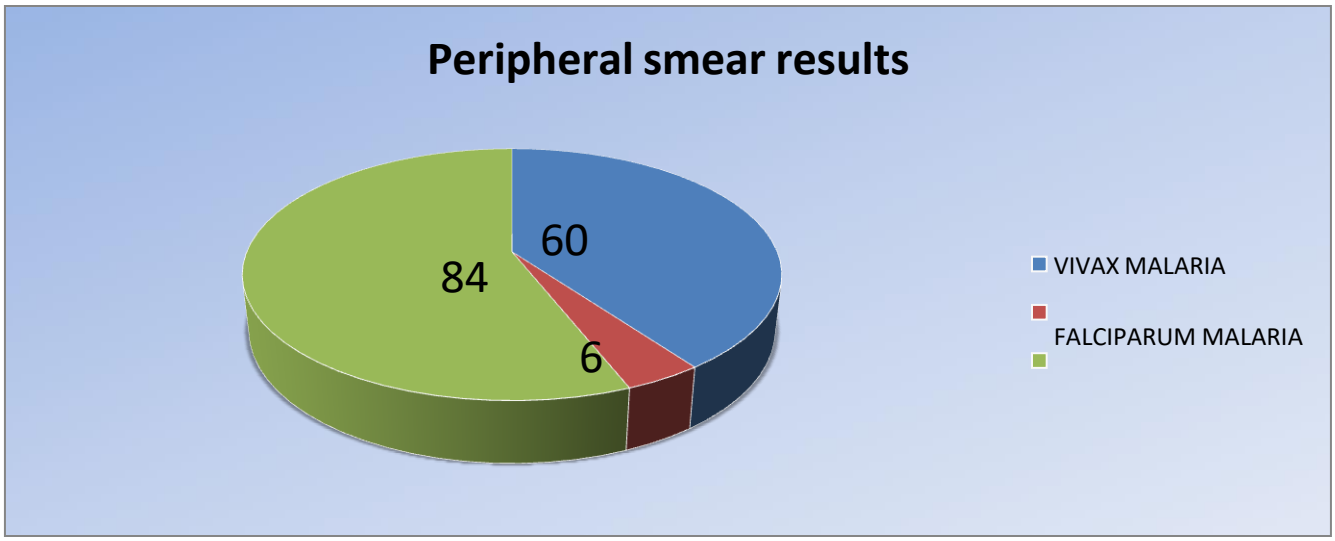

Graph 5: Patients with anemia (< 10 gm%):44(66.6%),Patients without anemia(>10gm%):22(33.4%)

**Graph 6: Platelet count**




Patients with platelet count <1.5 lakh: 38 (57.5%) Patients with platelet count >1.5 lakh: 28(42.5%)

**Graph 7, 8, 9: Diagnostic evaluation of QBC, RDT (pLDH) in comparison with peripheral smear study**



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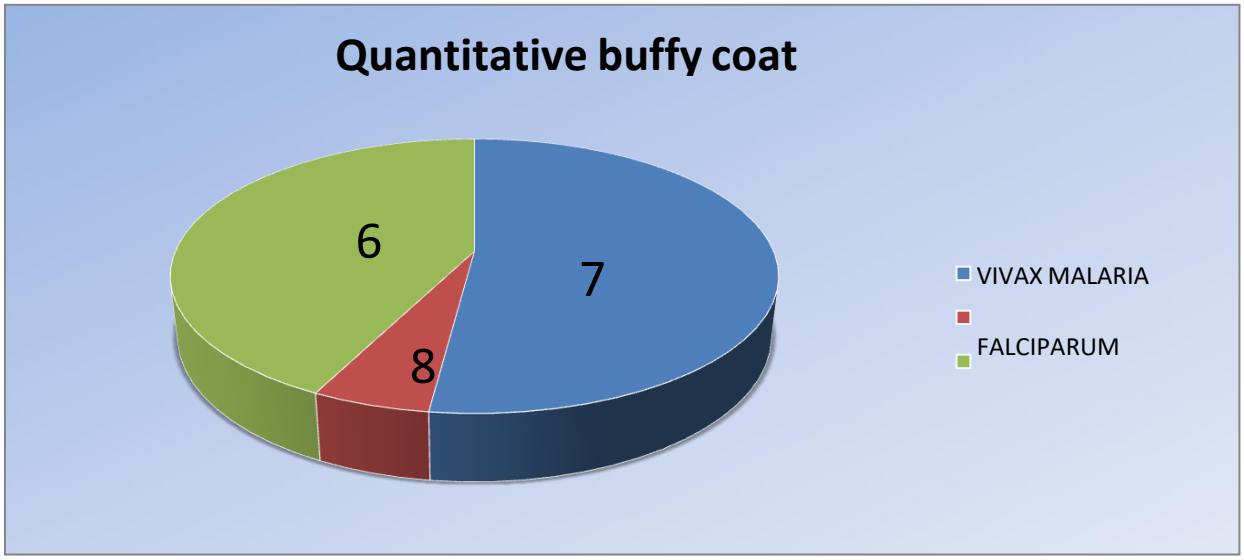


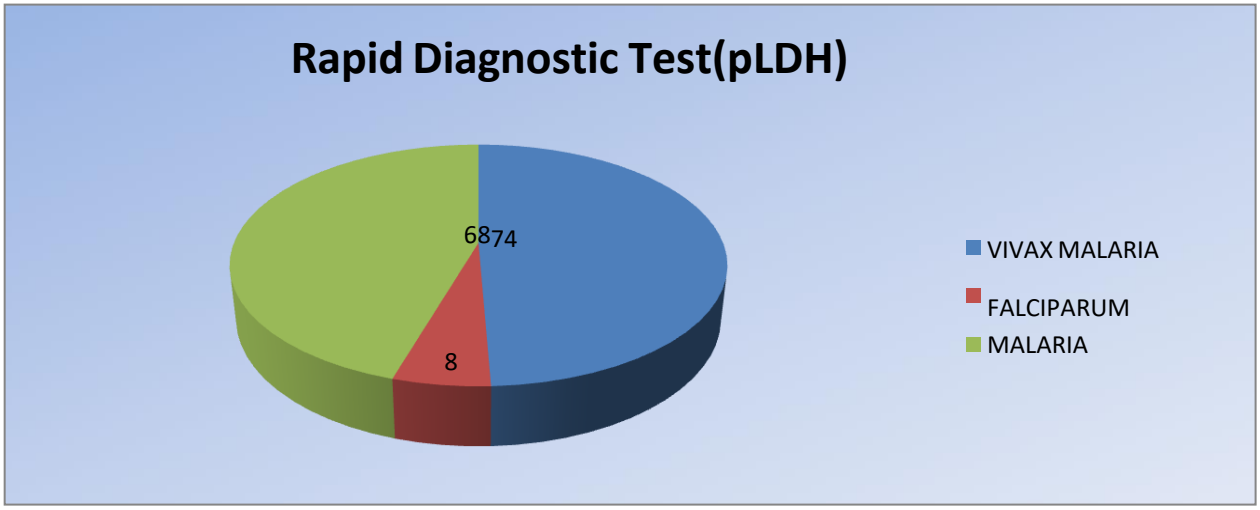


**DISCUSSION**

In our study, clinical suspicion of malaria was made in 150 children. Among them, only 66 were diagnosed to have malaria based on smear positivity. As the symptoms and the signs of malaria are vague, the clinical diagnosis of malaria is very difficult, and sometimes the over-enthusiastic diagnosis made on clinical grounds leads to unnecessary use of antimalarials, the major cause of emerging drug resistance. So we have to depend on laboratory investigations for accurate diagnosis [9]. Majority of cases of malaria in our study were between 6 months to 5 years of age group, which coincides with the study of Joshi H, et.al Fever was present in all (100%) cases of confirmed malaria on the day of admission. The characteristic fever with chills and rigor was observed in 19.7% of patients only. Next to fever, the commonest complaint is GIT symptoms (40.9%) in the form of nausea, vomiting, anorexia, abdominal pain, and loss of stools.16.7% of cases had a headache and 15.2% had muscle ache.2(8.3%) patients had seizures. Both were turned out to have falciparum malaria. On examination, pallor was observed clinically in 36 (54.5%) cases. Icterus was noted in 3 (4.5%) cases. This is due to hemolysis as evidenced by low hemoglobin levels in them.8 (12.1%) patients had hepatomegaly,14 (21.2%) had splenomegaly,36(54.5%) had hepatosplenomegaly [10]. The majority of children with malaria in our study, had GIT symptoms, pallor, and hepatosplenomegaly in addition to the fever which was similar to the earlier observation by Kochar DK et. al among the malaria cases, 66.6% of patients had hemoglobin levels of 7 -10 gm% and 15.1% of cases had <7 gm% [11]. In our study, the incidence of anemia in malaria detected by peripheral smear is more when compared to the study

by Limaye CS et.al whose predominant study population with malaria was between 9-12 years of age [12]. The increased incidence of anemia in our series may be explained by the superadded nutritional anemia observed in younger children. Among the malaria cases detected by smear, 57.5% had a platelet count of <1.5 lakh which is similar to the study observed by Mehta KS, et.al Of the 150 cases, 66(44%) cases were positive for malaria and 84(56%) cases were negative for malaria by peripheral smear microscopy. Out of 66 malaria cases, 60 (90.1%) were positive for *P. vivax* & 6 were positive for *P. falciparum*( 9.9%) .

**Results of Peripheral smear Vs RDT (*P. vivax* & *P. falciparum*)**

| | Estimate | 95% Confidence Interval |
|---|---|---|
| Sensitivity | 98.48% | 91.9- 99.73 |
| Specificity | 79.76% | 69.96- 86.96 |
| Positive predictive value | 79.27% | 69.28-86.63 |
| Negative predictive value | 98.53% | 92.13-99.74 |
| Diagnostic accuracy | 88% | 81.83-92.27 |
| Liklihood ratio of positive test | 4.86 | 4.33-5.46 |
| Liklihood ratio of negativetest | 0.019 | 0.002-0.136 |

**Results of Peripheral smear Vs QBC (*P. vivax* & *P. falciparum*)**

| | Estimate | 95% Confidence Interval |
|---|---|---|
| Sensitivity | 100% | 94.5- 100 |
| Specificity | 76.19% | 66.06-84.03 |
| Positive predictive value | 76.74% | 69.79-84.41 |
| Negative predictive value | 100% | 94.34-100 |
| Diagnostic accuracy | 86.67% | 80.3-91.2 |
| Liklihood ratio of positive test | 4.2 | 3.81-4.63 |
| Liklihood ratio of negativetest | 0.0 | 0.0 |

QBC test was positive in 86 cases and RDT (pLDH) was positive in 82 cases [13]. The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratios, and diagnostic accuracy of QBC and RDT for *P. vivax*, *P. falciparum* was compared with peripheral smear. The QBC detected 86 cases of malaria of which, 78 (90.7%) were positive for *P. vivax* and 8 (9.3%) were positive for *P. falciparum*. The cases which were positive by smear were also detected by the QBC method [14]. In addition, 20 cases that were not detected by smear, were diagnosed as malaria by QBC technique which includes 2 *P. falciparum* cases also. All patients who were malaria parasite negative by QBC method were also smear negative. The QBC method showed sensitivity, specificity, positive predictive value and negative predictive value of 100%,80%,76.92%,100% for *P. vivax* and 100%,98.61%,75%,100% for *P. falciparum*. The positive likelihood ratio for detection of *P. vivax* is 5 and for falciparum, it is 72. The negative likelihood ratio for detection of *P. vivax* is 0 and for *P. falciparum* also it is 0.0 [15]. The RDT(pLDH) test showed 82 cases were positive for malaria. Among the 82 cases, 74 (90.2%) were positive for *P. vivax* and 8 were positive for *P. falciparum* (9.8%). A case that was negative by RDT (pLDH) test was found to be positive by smear as well as QBC. This could be attributed to low antigen levels as observed by Murray CJ et al [16]. This test identified 17 additional cases of malaria which were negative by peripheral smear. All these 17 cases were also positive by QBC and showed a good response to antimalarials. Two cases of *P. falciparum* were missed by the peripheral smear method, found to be positive by both QBC and RDT methods. No mixed

infection was identified by any of these methods. No mortality has been observed in our study [17]. The positive likelihood ratio for detection of *P. vivax* is 5.9 and for falciparum, it is 72. The negative likelihood ratio for detection of *P. vivax* is 0.02 & for falciparum also it is 0.0 [18].

## CONCLUSION

Fever with chills, the classical feature of malaria was present in only 19.1% of malaria cases detected by peripheral smear. Next to fever, GIT symptoms were predominant. Among the malaria cases detected by peripheral smear, 66.6% had anemia and 57.5% had thrombocytopenia. Peripheral (thick and thin) blood smear examination is considered to be the gold standard for the diagnosis of malaria. In setups where the technicians are overloaded with hundreds of samples per day, the chance of missing the smear with a low parasite count is more. QBC is a much simpler, rapid, and highly sensitive diagnostic test to detect both vivax and falciparum malaria. The only disadvantage of QBC is the requirement for specialized instrumentation. RDT (pLDH) is highly sensitive in picking up falciparum cases, which is equal to that of QBC. So, it can represent a diagnostic tool for falciparum malaria where expert microscopy/QBC is not available. The sensitivity of RDT (pLDH) in diagnosing vivax malaria is much lower than the peripheral smear microscopy and QBC. In future the peripheral smear examination can be replaced by Quantitative Buffy Coat technique for the diagnosis of malaria. In resource limited settings where specialized instrumentation facilities are not available RDT (pLDH) can be used to diagnose malaria.

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