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Assessment of Methods of Diagnosis and Haematological Parameters in Malaria: A Hospital Based Study in Western Odisha

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Abstract:
Background: Malaria remains a substantial health problem in many parts of world, especially in the tropical developing nations. Management of malaria requires early and accurate diagnosis. The aim of this study was to measure and compare the diagnostic accuracy of different methods of diagnosis of malaria and to study the limitations mostly due to low parasite density. We also evaluate the haematological changes in malaria patients. *Material and methods:* The study was conducted on blood samples of patients presenting with pyrexia, attending the various Departments of VSS Institute of Medical Science &Research, Burla, Sambalpur. Blood samples were subjected to thick and thin blood smear examination, QBC method and Rapid Diagnostic Tests (RDTs). Haematological parameters including haemoglobin, total leukocyte count, differential count and 86.66% whereas *P. vivax* and mixed infections constitute 8.57% and 4.77% respectively of total malaria. The sensitivity of QBC test approached 100% with parasite densities above 500/cu.mm. Antigen detection method was found to be highly sensitive (92.70%) for the diagnosis of *P. falciparum* infection. Anemia was found in 62.86% and thrombocytopenia in 64.75% cases. *Conclusion:* QBC method is an important tool for the diagnosis of malaria particularly in the hospital environment. In the field and in emergency RDTs can be employed as a simple and easy tool. Anemia with thrombocytopenia may be important predictors of malaria infection in a acute febrile cases.

Keywords: Malaria, QBC, RDT, Anemia, Thrombocytopenia.

INTRODUCTIONS

Malaria continues to be a major health problem in some of the most populated areas of the world. It is one of the important causes of febrile illnesses in our part of the world. One of the most prevalent human infections worldwide, malaria results in 225 million cases each year. [1] Around 40% of the global population at risk of malaria resides in the South-East Asian Region. In the Indian subcontinent, distribution is heterogenous and governed by many climatic and physiological risk factors. [2] It is caused by protozoa parasite of the genus plasmodium which infects and destroys red blood cells. Four species of plasmodia (*P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax*) cause malaria in humans of which *P. falciparum* is the cause of morbidity and mortality. [3] However, *Plasmodium vivax* is the major malarial parasite in India, contributing towards the majority of cases. [4]

The clinical diagnosis of malaria is challenging because of the non-specific nature of the signs and symptoms, which overlap considerably with other febrile illnesses common in tropical regions. [5] This impairs diagnostic specificity and often promotes the indiscriminate use of antimalarials. As parasites of the blood for the majority of their complex life cycle, they expectedly induce hematological alterations. [6] Hematological abnormalities are considered a hallmark of malaria and statistical analyses have shown that many of these hematological values may lead to an increased clinical suspicion for malaria, thus initiating a prompt institution of specific therapy even in the absence of a positive smear report for malaria. [7]

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The hematological abnormalities that have been reported to invariably accompany infection with malaria include anemia, thrombocytopenia, splenomegaly, mild-to-moderate atypical lymphocytosis and rarely disseminated intravascular coagulation (DIC). [8] There have also been reports of leucopenia and leucocytosis. [9]

Hematological changes are among the most common complications encountered in malaria. [10] Prediction of the hematological changes enables the clinician to establish an effective and early therapeutic intervention in order to prevent the occurrence of major complications. These parameters are measurable indices of blood that serve as a marker for disease diagnosis. [11] Abnormalities such as anemia and thrombocytopenia have been observed in patients with malaria. [12-17] In this study, we analyzed and statistically evaluated the hematological changes in cases of malaria and whether they could guide physicians to institute specific antimalarial treatment.

MATERIAL AND METHODS:

The present study was done in the department of Pathology and Central Laboratory, VSS Institute of Medical Science & Research, Burla, Sambalpur, Odisha over a period of 3 years from February 2013 to February 2016. The study was undertaken after approval by institutional ethics committee (IEC). Informed consent was obtained from all included patients. About 2 ml venous blood was collected in sterile EDTA vacutainer tube from patients presenting with pyrexia, attending the various Departments. Blood samples were subjected to thick and thin smear examination, antigen detection - as per kit instruction and QBC method. Thick blood smear was stained with Jaswant Singh Bhattacharya (JSB) stain and thin smear was stained with Leishman's stain.

The parasite density, expressed as the number of parasites per microL of blood, was calculated by dividing the number of parasites by the number of WBCs counted and then multiplying it by an assumed WBC density of 6000 microL. We graded the parasite count as 1+, 2+, 3+ and 4+ for <100/microL, 100 – 500/microL, 501 – 1000/microL and >1000/microL respectively. The QBC technique using microhematocrit tubes which have the acridine orange, fluorescence from malaria parasites and detect through an epi-fluorescent microscopy. An attempt was made to estimate the relative quantity of parasites in the specimen using the plus systems as 1+, 2+, 3+ and 4+ for <1 parasite per QBC field, 1-10 parasites per QBC field, 11-100 parasites per QBC field and >100 parasites per QBC field respectively. Malascan Rapid test for malaria *Pf/Pan* Zephyr Biomedical, India was used to diagnose malaria infection. This malaria RDT targets the detection of histidine rich protein-2 (HRP-2) antigen of *plasmodium falciparum* and Pan Specific Aldolase of non *falciparum* species. Hematoloical parameters of malaria patients, evaluated using SYSMEX XN1000, CBC counter provide data on WBCs, Hb level, Total platelet count, MCV, MCH, RDW and differential count.

Statistical Analysis

The sensitivity, specificity, positive predictive value(PPV),negative predictive value(NPV) of thin smear, QBC and antigen detection methods were determined using the software statistical package for the social sciences (SPSS) version 2016.

RESULTS:

A total of 186 samples of febrile patients were observed over the period of study. Of these a total of 105 samples were positive by microscopy in JSB stained thick smear**.** Males constitute 68 (64.76%) of malaria cases whereas females constitute 37 (35.24%) of total number of malaria cases. Most of the patients were in the age-group of 26 – 35 years which constitutes 28 (22.66%) of all cases. In almost all the age-groups males constituted more number of cases than females. JSB stained thick smears showed plenty of trophozoites (ring form) of *P.falciparum*[Fig1a]Thin smears showed sickle shaped gametocyte of *P.falciparum*[Fig1b].Leishman stained peripheral smears showed ring form of malaria, many Red cells infected with trophozoites and Schizonts forms of *P vivax*[Fig 1c]. High degree of parasitemia was seen in RBC infected with *P.Falciparum* in QBC.[Fig 1(d].*P.falciparum* constitute 91 (86.66%) of the total number of malaria cases whereas *P. vivax* and mixed infections constitute 09 (8.57%) and 05 (4.77%)respectively[]

Out of 105 thick smear positive sample, 84 (80.00%) were positive by thin smear, 97 (92.38%) were positive by antigen detection (RDT) and QBC was positive in 92 (87.61%) sample.81 samples out of total 186 samples were negative by thick smear. Out of 81 such samples 5 samples were positive by QBC method. Subsequently these 5 samples found to be negative for thick, thin and antigen detection test. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), of thin smear, QBC, and RDT methods were compared with thick smear as gold standard. Measure of sensitivity, specificity, PPV, NPV of thin smear was 80, 100, 100, 82.92 respectively, for QBC it was 87.61, 93.82, 94.84, 87.85 respectively and 92.38, 100, 100, 91.75 respectively represent for RDT[Table1a].

Sensitivity of QBC and RDT with respect to species identification was calculated. *P. faciparum*, *P. vivax* and mixed infection the sensitivity of QBC was 89.01, 77.78, 80.00 respectively [Table1b]. For RDT the sensitivity of *P.falciparum* and non-falciparum was 92.70 and 88.89 respectively[Table1c]. Mixed infection was taken as *P. falciparum* infection in RDT as differentiation not possible. Antigen detection method was found to be highly sensitive (92.70%) for the

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diagnosis of *P. falciparum* infection. Evaluation of QBC and RDT test in detection of different levels of parasitaemia was done. The sensitivity ofQBC test approached 100% with parasite densities above 500/microL of blood[Table1d]. The sensitivity of antigen detection test approached 100% with parasite densities above 100/microL of blood[Table1e].

In this study haematological abnormalities mostly anemia and thrombocytopenia was observed in malaria infection [Fig2a&Fig 2b].Leucocytosis observed in 26 (24.76%) cases with neutrophilia in 18 (17.14%) cases. Anemia was observed in 66 (62.86%) cases with 42 (40%) had mild anemia[Table 2a]. 68 (64.75%) cases had thrombocytopenia with 45 (42.86%) had mild degree of thrombocytopenia i. e. TPC between 1 to 1.5 lacs/cu.mm [Table 2b].

Table1a .Comparision of diagnostic test when peripheral thick blood smear was adopted as gold standard

Methods of diagnosis of Malaria	Sensitivity(%)	Specificity (%)	PPV(%)	NPV(%)
Thinsmear	80	100	100	82.92
QBC	87.61	93.82	94.84	87.85
RDT	92.38	100	100	91.75

Table1b. Sensitivity of QBC for species identification

Species of Malaria	No of cases	No of QBC +VE	Sensitivity(%)
P. falciparum	91	81	89.01
P.Vivax	09	07	77.78
Mixed infection (Pf+Pv)	05	04	80.00B

Table1c. Sensitivity of RDT For species identification

Species of Malaria	No of cases	No of RDT +VE	Sensitivity(%)
P. falciparum	96	89	92.70
Non Falciparum	09	08	88.89B

Table1d. Sensitivity of QBC in different levels of Parasitemia

Parasite Density	Smear +ve cases	QBC +VE cases	Sensitivity(%)
<100/µL	35	24	68.57
100 - 500/µL	52	50	96.15
501 - 1000/µL	10	10	100
>1000/µL	08	08	100

Table1e. Sensitivity of RDTs in different levels of Parasitemia

Parasite Density	Smear +ve cases	RDT +VE cases	Sensitivity(%)
<100/µL	35	27	77.14
100 - 500/µL	52	52	100
501 - 1000/µL	10	10	100
>1000/µL	08	08	100

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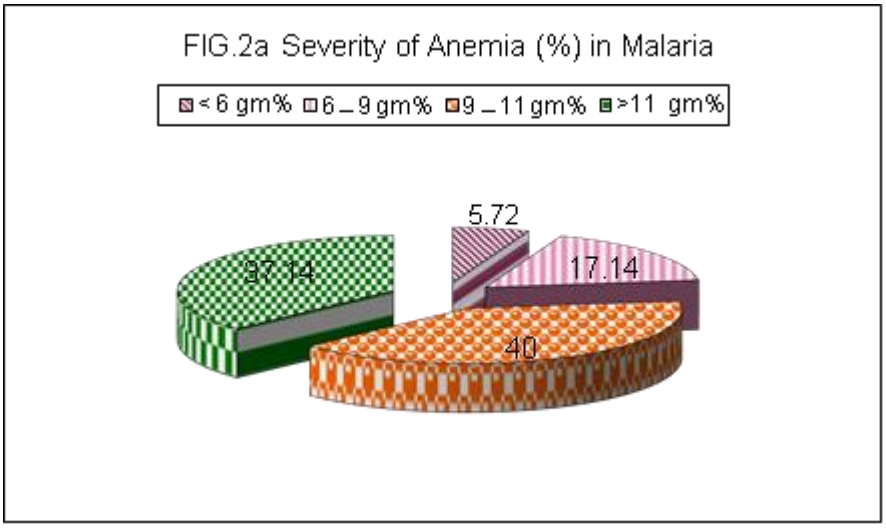

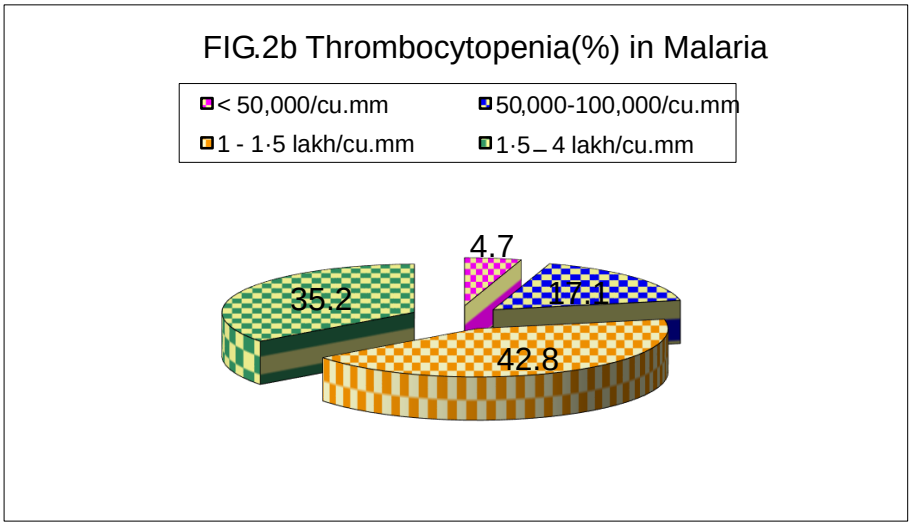

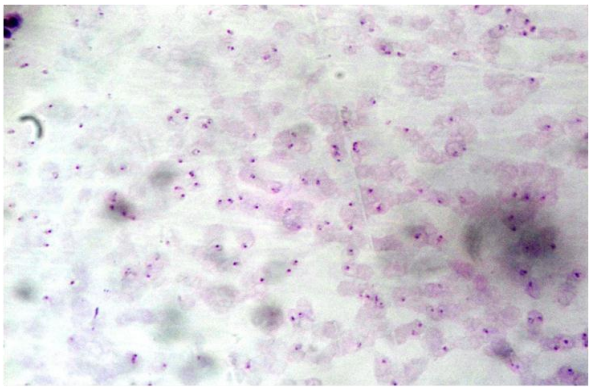

Fig 1(a)Thick blood film (dehaemoglobinised) showing trophozoites (ringform)of *Plasmodium falciparum*. (JSB, X500)

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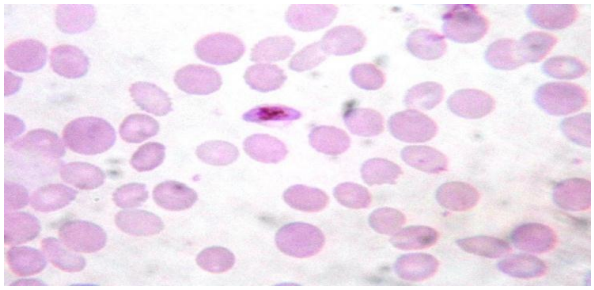

Fig 1(b)Thin blood film showing sickle shaped gametocyte of *Plasmodium falciparum* (Leishman, X500)

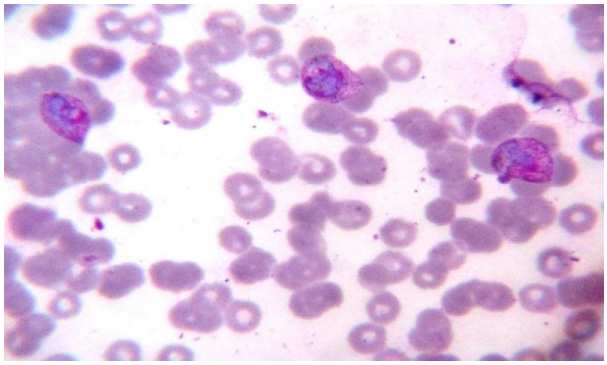

Fig 1(c)Thin blood film showing young ameboidtrophozoite and early schizonts of *Plasmodium vivax*

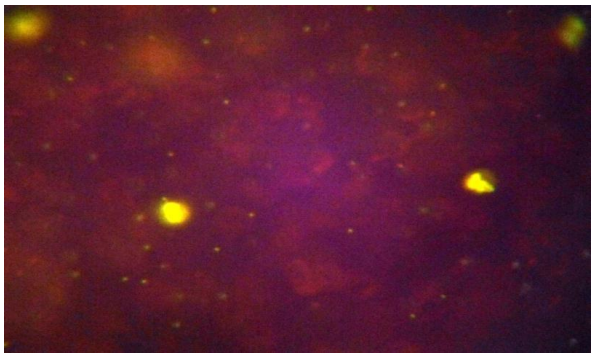

Fig 1(d)QBC film showing trophozoites of *Plasmodium falciparum*.

Table 2a. Severity of anaemia in malaria

Degree of anaemia (Hb%)	No. of cases			percentage
	Pf	*Pv*	*Pf+Pv*	
< 6 gm%	06	00	00	05.72
6 – 9 gm%	15	02	01	17.14
9 – 11 gm%	38	03	01	40.00
>11 gm%	33	04	02	37.14

Table 2a. Relationship Of Platelet Count With Malaria

Degree of thrombocytopenia (TPC)	No. of cases			percentage
	Pf	*Pv*	*Pf+Pv*	
< 50,000/cu.mm	05	00	00	04.76
50,000-100,000/cu.mm	17	01	00	17.14
1 - 1·5 lakh/cu.mm	40	04	01	42.85
1·5 – 4 lakh/cu.mm	32	03	02	35.25

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DISCUSSION:
Most of the malaria cases are reported from rural and tribal areas of India. Tribal population constituting 8.6% of total population account for more than 50% of total malaria cases and about 50% death due to malaria in the country[2]Malaria diagnosis is challenging task in rural and tribal areas because of poor healthcare infrastructure. The reluctance of these tribal individual to seek medical care further complicate the issue of malaria diagnosis in tribal areas. We have evaluated the sensitivity, specificity, PPV, NPV of QBC and RDT method when JSB stained thick smear was adopted as gold standard.

Out of a total number of 105 positive cases 91 patients (86·66%) had *falciparum* malaria, 9 patients (8.57%) had *vivax* malaria and 5 (4.77%) had mixed infection. This observation reflects the fact that the maximum number of malaria cases came to the hospital are cases of *falciparum* malaria. Since *falciparum* malaria gives rise to various complications which required admission to the hospital, the percentage of *falciparum* malaria cases in this hospital-based study out numbered that of *vivax* malaria. The sensitivity of Leishman-stained thin smear was found to be 80%, specificity and positive predictive value were 100% each. The sensitivity was higher and specificity was comparable to what as reported else[18]. The sensitivity of the QBC has been reported as high as 99.70% by Benito a *et al*[19]and relatively low sensitivity 78·94% has observed by Parija SC *et al* [18]. One of the reasons for this low sensitivity could be that as the hospital is present in an endemic region for malaria the levels of parasitemia could have been low. The specificity of the QBC test as reported by previous study was 98%by Parija SC *et al* & Bosch I *et al* [18,20].

The sensitivity and specificity of the QBC method for detection of malaria parasite in this study turned out to be 87.60% and 93.82% respectively. There were five cases found to be positive by QBC, which were subsequently found to be negative for thick, thin and antigen detection test. This can be explained by the fact that certain artefacts in blood like Howell Jolly bodies, normoblast or platelet fragments might resemble the ring forms of malariaparasites. The sensitivity of the QBC test increases significantly with a rise in the level of parasitemia. It is seen that with parasite density of less than 100/microL of blood the sensitivity of the QBC test is 68.14%, with parasite density of 100 – 5 00/microL of blood it is 96.15% and it is 100% with parasite densities above 500/microL of blood. Antigen detection method for diagnosis of malaria had a sensitivity, specificity and PPV of 92.38%, 100% and 100% respectively. This test was based on detection of HRP-2 and aldolase with the help of monoclonal antibodies. The sensitivity obtained for this kit-based procedure was close to those observed on similar principle[21,22].

The low sensitivity could be attributed to low parasitemia levels as observed by study who observed 75% sensitivity at parasitemia<100/µL[23]. The specificity was comparable to other observers using the tests based on similar principle[24,25].The sensitivity of the antigen detection test increases significantly with a rise in the level of parasitemia. It is seen that with parasite density of less than 100/microLof blood the sensitivity of the RDT is 77.14%, with parasite density above 100/microL of blood it was 100%. So the test was found to be user friendly and interpretation was more objective as compared to smear and QBC.

Anaemia is an inevitable consequence of malaria infection and its degree is related to the density of parasitemia and to the presence of severe manifestations. Haemoglobin values encountered in *Plasmodium falciparum* infections are significantly lower than in *vivax* malaria due to greater proportion of parasitized erythrocytes, shortening of life span of RBC's as well as due to ineffective erythropoiesis. It is difficult to attribute the cause of anaemia solely due to malaria, because of associated Iron, folic acid and other nutritional deficiencies in the endemic areas. We have found anaemia in 62.86% and thrombocytopenia in 64.75% of malaria cases . This incidence was comparable with other author's observations[26,27]. Leucopenia was frequently seen in malaria infected patients which was confirmed by studies that have demonstrated leucopenia [28]. and contrast with other study that had demonstrate leucocytosis [17].We have found leucocytosis and leucopenia in 24.76% and 5.71% respectively of malaria patients in our study. Malaria has a significant impact on haematological profile, most marked being thrombocytopenia and anaemia. Out of the infection types prevalent in our part of world *falciparum* malaria causes maximum drop in platelet count and haemoglobin levels.

Limitations
Comparative evaluation with newer techniques like LAMP, Flow cytometry, PCR &Automated Blood cell counter could not be done as the facility was not available during the study period.

CONCLUSION:
From present study it can be concluded that *Plasmodium falciparum* was the predominant species detected in the patients of malaria. Though the QBC method has a few limitations like high cost and relative insensitivity when used in field conditions, the speed and ease of method and its excellent overall sensitivity, particularly in the hospital environment, makes it an important tool for the diagnosis of malaria. Situation, where adequate back up is not available and in emergency simpler and easy to use antigen detection technique, can be employed. Peripheral smear is ideal for species identification, parasite counting and confirmation of malaria. Malaria parasites exhibit important changes in many

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haematological parameters, most commonly changed parameters were anemia and thrombocytopenia and it may be important predictors of malaria infection in a acute febrile cases.

REFERENCES:

1. World Health Organisation (WHO), World malaria report 2018.who.int/iris/bit-stream/handle/10665/275867/9789241565653-eng. pdf [cited 2019 April14].
2. Sharma RK,ThakorHG,SahaKB,SonalGS,DhariwalAC,SinghN.Malaria situation in India with special reference to tribal areas. Indian J Med Res 2015;141:537.
3. Pradhan A, Anasuya A, Pradhan MM, Ak K, Kar P, Sahoo KC, *et al*. Trends in malaria in Odisha, India-An analysis of the 2003–2013 time-series data from the National Vector Borne Disease Control Program. PLoS One. 2016; 11:e0149126. https://doi.org/10.1371/journal.pone.0149126 PMID: 26866696
4. Chotivanich K, Silamut K, Day NPJ. Laboratory diagnosis of malaria infection-a short review of methods. Aust J Med Sci. 2006;27:11–15.
5. MukrySN,SaudM,SufaidaG,ShaikhK,NazA,Shamsi TS. Laboratory diagnosis of malaria: comparison of manual and automated diagnostic tests. Can J Infect Dis Med Microbiol2017;2017:9286392.
6. Notomi T, Okayama H, Masubuchi H, *et al*. Loop-mediated isothermal amplification of DNA. Nucleic Acids Res 2000;28:e63.
7. Wongchotigul V, Suwanna N, Krudsood S, Chindanond D, Kano S, Hanaoka N, Akai Y, Maekawa Y, Nakayama S, Kojima S, Looareesuwan S. The use of flow cytometry as a diagnostic test for malaria parasites. Southeast Asian J Trop Med Public Health.2004;35:552–559.
8. Morassin B, Fabre R, Berry A, Magnaval JF. One year's experience with the polymerase chain reaction as a routine method for the diagnosis of imported malaria. Am J Trop Med Hyg.2002;66:503–508.
9. Hanscheid T, Melo-Cristino J, Pinto BG. Automated detection of malaria pigment in white blood cells for the diagnosis of malaria in Portugal. Am J Trop Med Hyg. 2001;64:290–292.
10. Adeoye GO, Nga IC. Comparison of Quantitative Buffy Coat technique (QBC) with Giemsa-stained Thick Film (GTF) for diagnosis of malaria. Parasitol Int. 2007;56:308–312.
11. Wongsrichanalai, C., M.J. Barcus, S. Muth, A. Sutamihardja and W.H. Wernsdorfer, 2007. A review of malaria diagnostic tools: Microscopy and rapid diagnostic test (RDT).anthones inhibitors of α-glucosidase and glycation from *Garcinia nobilis*. Am. J. Trop. Med. Hygiene, 77: 119-127.
12. Batwala, V., P. Magnussen, K.S. Hansen and F. Nuwaha, 2011. Costeffectiveness of malaria microscopy and rapid diagnostic tests versus presumptive diagnosis: Implications for malaria control in Uganda. Malaria J., Vol. 10.
13. Surve KM, Kulkarni AS, Rathod SG, Bindu RS. Study of haematological parameters in malaria.Int J Res Med Sci. 2017;5(6)2552-2557. doi: [Article:http://dx.doi.org/10.18203/2320- 6012.ijrms20172446][Crossref]
14. Rojanasthein S, Surakomolleart V, Boonpucknavig S, Isarangkura P. Hematological and coagulation studies in malaria. J Med Assoc Thai. 1992;75(1)190-194. [Crossref]
15. Sharma SK, Das RK, Das BK, Das PK. Haematological and coagulation profile in acute falciparum malaria. J Assoc physicians India. 1992;40(9)581-583. [Crossref]
16. Ladhani S, Lowe B, Cole AO, Kowuondo K, Newton CR. Changes in white blood cells and platelets in children with falciparum malaria: relationship to disease outcome. Br J Haematol.2002;119:839–847.
17. Maina RN, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L, Jones D, Ogutu BR. Impact of Plasmodium falciparum infection on haematological parameters in children living in Western Kenya. Malar J. 2010;9(Suppl 3):S4.
18. Parija SC, Dhodapkar R, Elongovan S, Chaya DR. A comparative study of blood smear, QBC and antigen detection for diagnosis of malaria. Indian J PatholMicrobiol 2008 ; 51 108-12.
19. Benito A., Roche J., Molina R., Amela C. & Alvar J. (1994) Application and evaluation of QBC® malaria diagnosis in a holoendemic area. Applied Parasitology 35, 266–272.
20. Bosch I, Bracho C, Perez HA. Diagnosis of malaria by acridine orange flurescent microscopy in an endemic area of Venezuela. Mem Inst Oswaldo Cruz 1996; 91:83-6.
21. Balakrishna HT, Saldanha P, Khadilkar U. Diagnostic accuracy of antigen capture dip-stick assay in *falciparum* malarial infection. Indian journal of medical science 2000; 54: 227-232
22. Nicastri, E., N. Bevilacqua, M.S. Schepisi, M.G. Paglia and S. Meschi *et al*., 2009. Accuracy of malaria diagnosis by microscopy, rapid diagnostic test and PCR methods and evidence of antimalarial overprescription in non-severe febrile patients in two Tanzanian hospitals. Am. J. Trop. Med. Hygiene, 80: 712-717.
23. Iqbal J, Sher A, Hira PR Al-Owaish R. Comparison of the OptiMAL test with PCR for diagnosis of malaria in immigrants. J Clin Microbiol 1999; 39 : 36446.
24. Iqbal J, Hira PR, Sher A, AI-Enezi AA. Diagnosis of imported malaria by *Plasmodium* lactate dehydrogenase (pLDH) and histidine-rich protein 2 (PfHRP-2) based immunocapture assays. Am J Trop Med Hyg2001;64:20-3.
25. Palmer C.J., Lindo J.F., Klaskala W.I., Quesada J., Kaminsky R. & Ager A.L. (1998) Evaluation of Journal of Clinical Microbiology 36,203-206

ISSN: 0975-3583, 0976-2833 VOL14, ISSUE8, 2023

26. Erhabor O, Mohammad HJ,OnuiguiFU,AbdulrahamY,Ezimah AC Anaemia and Thrombocytopenia among Malaria Parasitized Children in Sokoto, North North Western Nigeria. J HematolTransfus 2(2): 1020 (2014).
27. Lathia TB, Joshi R. Can haematological parameters discriminate malaria from nonmalarious acute febrile illness in the tropics ? India J Med Sci. 2004; 58: 239-244.
28. Erhart LM, Yingyuen K, Chuanak N, Buathong N, Laoboonchai A, Miller RS, Meshnick SR, Gasser RA Jr, Wongsrichanalai C. Hematologic and clinical indices of malaria in a semi-immune population of western Thailand. Am J Trop Med Hyg.2004; 70:8–14.