

Clinical Profile of Malaria in and around Hubballi-Dharwad: A Region of North Karnataka

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ABSTRACT

Introduction: Malaria is an endemic vector borne parasitic infection. *Plasmodium vivax* has been associated with severe malaria while *P. falciparum* is traditionally associated with severe course. Of late, *P. vivax* is increasingly reported to cause severe and life threatening disease. However, majority of *P. vivax* are sensitive to antimalarials and therefore, it is important to speculate this pathogen.

Aim: To study the clinical profile of confirmed malaria cases.

Materials and Methods: This prospective study was undertaken at SDM College of Medical Sciences and Hospital, Dharwad, Karnataka, India, between the period of 2010 to 2012 for the duration of two years. A total of 124 clinically suspected malaria cases aged from 8 years to 65 years were included in the study. Laboratory identification was done by Quantitative Buffy Coat (QBC). A comparative analysis of clinical presentations in 62 QBC positive samples and an equal number of age and sex matched QBC negative was done.

Results: Out of 62 QBC positive samples, *Plasmodium vivax* was seen in 40/62 (64.52%) patients while *P. falciparum* in 10/40 (16.13%) cases. Mixed infection by *P. vivax* and *P. falciparum* was seen in 12/40 (19.35%) cases. Fever, chills and

headache were common symptoms. Pallor was seen in 23/40 (37.1%) cases and icterus, splenomegaly and vomiting were seen in 14/62 (22.6%) cases followed by hepatosplenomegaly in 11 (17.7%) cases. Among QBC negative controls, fever (100%), chills 51/62 (82.3%), rigors 21/62 (33.9%) and pain abdomen 15/62 (24.2 %) were the common symptoms. Pallor and hepatomegaly was seen in 19.4 % and 11.3% respectively among the QBC negatives. Ten out of 11 (90.9%) of females and 37/51 (72.5%) of males suffering from malaria had anaemia. Thrombocytopenia was seen in 59/62 (95.2%) cases of which 33 cases had moderate thrombocytopenia (53.2%) while 17 cases had severe thrombocytopenia. In QBC negative controls, severe thrombocytopenia was noted in 4 (6.5%) samples, mild and moderate thrombocytopenia was seen in 14 and 16 (22.5 and 25.8%) patients respectively. About 94% cases recovered completely. One patient suffering from *P. vivax* succumbed to the infection.

Conclusion: *Plasmodium vivax*, traditionally thought to cause benign malaria can also produce life threatening complications similar to falciparum malaria. Early recognition of signs and symptoms of severe malaria and laboratory confirmation of species is most important in management of this condition.

Keywords: Icterus, Quantitative buffy coat, Splenomegaly, Vivax

INTRODUCTION

Malaria, an important mosquito borne parasitic infection, remains a major cause of morbidity and mortality in India [1]. *Plasmodium vivax* is the most prevalent human malarial parasite followed by *P. falciparum* [2]. India contributes 80% of cases in South East Asian Region [1]. In India, approximately 95% population lives in regions endemic for malaria [3].

As the clinical presentation of the disease is inconsistent, malaria presents a diagnostic challenge in most tropical countries. With the increase in drug resistance in *P. falciparum* in tropical regions, it is not easy to treat febrile patients empirically with low cost standard drugs. WHO recommends that malaria case management should be based on parasite

diagnosis in all cases, except in young children [4]. Therefore, high suspicion and laboratory confirmation are essential in the management and prevention of malaria cases.

Accurate diagnosis of malaria requires rapid, sensitive and specific tests that are available at affordable cost. Peripheral blood smear (PS) has remained the gold standard for the diagnosis of malaria. The most important limitation of this test is the need for an "expert" microscopist. Newer methods like Quantitative Buffy Coat (QBC), antigen detection by immunochromatography and Polymerase Chain Reaction (PCR) have enhanced the capability of laboratory in the diagnosis of malaria [5].

The QBC test is a concentration technique and quick method. In QBC, nucleic acid of malarial parasite is stained by

fluorescent dye. Maturation of parasite within the red blood cells reduces the buoyant density of infected erythrocyte. This unique phenomenon is basis of QBC technique for malaria diagnosis. This test is more useful as a screening test in laboratories having higher sample load and also in endemic areas where parasites levels are low [6].

The endemicity of malaria in our country and serious nature of this disease necessitates critical evaluation of clinical signs, symptoms and various laboratory methods for diagnosis of this potentially dangerous condition. This study was undertaken to study the various clinical signs and symptoms that helps to differentiate malaria from other acute febrile illness.

MATERIALS AND METHODS

The present prospective study was conducted in a Tertiary Care Hospital from September 2010 to May 2012 after obtaining permission from Institutional Ethical Committee. A total of 124 clinically malaria suspected cases aged from 8 years to 65 years were included in the study. For 62 QBC positive patients, age and sex matched 62 QBC negative cases were studied. Among the 124 cases studied, 51/62 were males and 11/62 were females in each group.

Inclusion criteria: The clinically suspected cases of malaria with fever that were positive by QBC were studied for different clinical manifestations. Both positive and negative cases were further evaluated for the various clinical signs and symptoms that patients presented with. Symptoms compared among cases and controls were fever, chills, headache, rigors, vomiting, and pain in abdomen. The signs compared were icterus, hepatomegaly, splenomegaly, pallor and altered sensorium.

Exclusion criteria: QBC positive patient with positive serology test for other infectious diseases were excluded from the study.

From each patient approximately 5 ml of blood sample was collected from cubital vein in EDTA bulb to perform QBC test after taking informed consent from all the patients

Clinical details: Details of the history and clinical examination of the cases and controls included in the study were recorded on a proforma. A sample positive by QBC test was reconfirmed by Leishman's stain stained thin peripheral blood smear from the same sample. Hemoglobin concentration, leukocyte count and platelet count of all the patients included in the study were noted in the proforma.

QBC method [7]: The QBC test was performed using QBC Malaria test (QBC Diagnostics Inc. Philipsburg, PA, USA). QBC microscope used was LABOMED Research Trinocular Microscope Lx 400 with epi illuminator with LED incident light for 50X objective. The labeled QBC malaria capillary tube was filled with 55 to 65 μ l of well mixed blood to a level between the blue lines and was rolled between the fingers to mix the blood with the anticoagulant and acridine orange coating. A plastic closure was applied to seal the tube at one end and a float was placed into the unsealed end of the tube using

a forceps. The tube was centrifuged at 10,000 rpm for 5 minutes. The QBC tube was observed under oil immersion with 50X objective in a para viewer with the minimum working distance of 0.34 mm at the interface of the granulocyte and red blood cell layers. Entire circumference of the tube was examined. Total examination time to interpret a negative result was approximately 5 minutes.

Different stages of different species of *Plasmodium* were identified using the description that appeared as follows.

Trophozoites: Distinct bicoloured signet ring forms strikingly apparent within RBCs near the granulocyte layer. The rings of *P. falciparum* were smaller compared to *P. vivax* and multiple ring forms were often present. Gametocytes of *P. falciparum* were observed as yellow crescent shaped bodies with brownish black pigment. Schizonts of *Plasmodium vivax* were recognized by the presence of dark brown malaria pigment.

STATISTICAL ANALYSIS

Statistical analysis of signs and symptoms among cases and controls and their degree of association was calculated by Kappa statistics using software SPSS Statistics 20.

RESULTS

Total of 124 patients were included in the study. Among the total 124 blood samples tested, QBC was positive in 62 patients.

In the 62 malaria case samples, *P. vivax* only was detected in 40 (64.5%) samples, while *P. falciparum* was seen in 10 (16.1%). An important third group of 12 (19.35%) cases was formed by samples showing mixed infections by *P. vivax* and *P. falciparum*. Of these 62 QBC positive cases, male patients accounted for 51/62 (82.3%) cases while the females accounted for only 11/62 (17.7%) of cases.

The age group included in the study ranged from 8 -65 years. Most commonly affected age group was 21-30 years with 45.2% positive cases [Table/Fig-1].

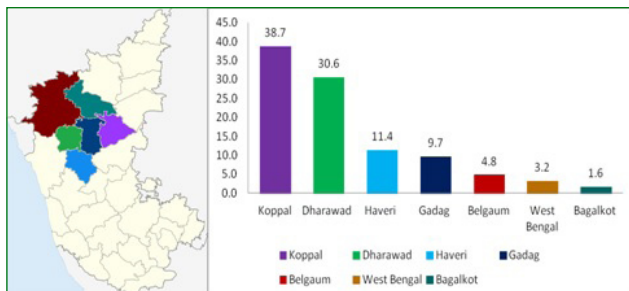
Maximum number of malaria cases were detected from Koppal district 24/62 (38.7%) followed by Dharwad district 19/62 (30.6%) [Table/Fig-2].

Fever was the most consistent symptom (however, fever was one of the inclusion criteria for the cases and controls). Chills and headache were found in 55/62 (88.7%) and 26/62 (41.9%) cases respectively and were the next common findings. Pallor was observed in 37.1% cases. Icterus, splenomegaly and vomiting were seen in 14 cases and hepatosplenomegaly in 11 cases. Among the QBC negative patients chills were seen in 51/62 (82.3%) cases followed by rigors 21/62 (33.9%), headache 18/62 (29%), pain in abdomen 15/62 (24.2) and pallor 12/62(19.4%) [Table/Fig-3,4].

Icterus and hepatosplenomegaly have better predictive efficacy in the diagnosis of malaria. Icterus had the positive predictive value of 87.5% while hepatosplenomegaly had positive predictive value 73.3%.

Age	Number	%
1-10	1	1.6
11-20	13	21.0
21-30	28	45.2
31-40	9	14.5
41-50	6	9.7
51-60	4	6.5
61-70	1	1.6
Total	62	100.0

[Table/Fig-1]: Depicting the age wise distribution of positive cases.



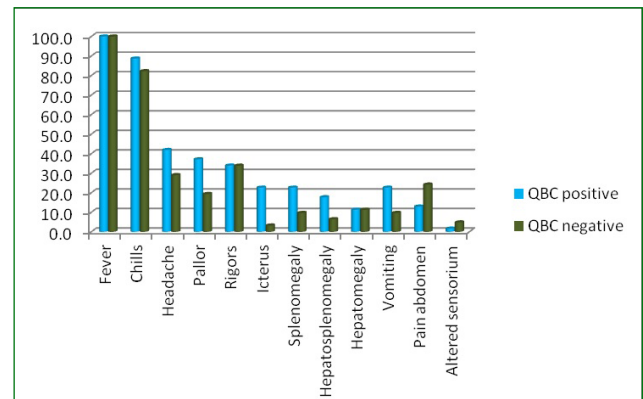
[Table/Fig-2]: Showing geographical distribution of cases.

Signs & Symptoms	QBC +ve	%	QBC neg	%
Fever	62	100.0	62	100.0
Chills	55	88.7	51	82.3
Headache	26	41.9	18	29.0
Pallor	23	37.1	12	19.4
Rigors	21	33.9	21	33.9
Icterus	14	22.6	2	3.2
Splenomegaly	14	22.6	6	9.7
Vomiting	14	22.6	6	9.7
Hepatosplenomegaly	11	17.7	4	6.5
Pain abdomen	8	12.9	15	24.2
Hepatomegaly	7	11.3	7	11.3
Altered sensorium	1	1.6	3	4.8

[Table/Fig-3]: showing signs and symptoms among QBC positive and QBC negatives.

In both the sexes, hemoglobin concentrations indicative of anaemia were closely associated with malaria. 90.9% (10/11) of females and 72.5% (37/51) of males suffering from malaria had anaemia. Anaemia was considered based on the hemoglobin values.

Thrombocytopenia in malaria has been classified into mild, moderate and severe type. In the present study, 59 (95.2%) cases showed thrombocytopenia of which 33 had moderate thrombocytopenia (53.2%) and 17 cases had severe thrombocytopenia. Among the QBC negative controls, severe thrombocytopenia was observed in 4 (6.5%) cases, mild and



[Table/Fig-4]: Distribution of signs and symptoms among malaria suspected cases.

Thrombocytopenia	Platelet count	No (QBC positive)	%	No (QBC negative)	%
Severe	<50,000	17	27.4	4	6.5
Moderate	50,000-1,00,000	33	53.2	16	25.8
Mild	1,00,000-1,50,000	9	14.5	14	22.5
No thrombocytopenia	>1,50,000	3	4.9	28	45.1
	Total	62	100	62	100

[Table/Fig-5]: Showing thrombocytopenia among the cases and controls.

moderate thrombocytopenia was seen in 14 and 16 (22.5 & 25.8%) patients respectively [Table/Fig-5].

It was interesting to see that 71% (44/62) of cases had normal range leucocyte count. In the control group, 9/62 (14.5%) showed leukocytosis and majority of them 43/62 (69.3%) had leucocytes within normal range.

All the cases received antimalarial treatment. Treatment included artesunate, chloroquine and quinine alone or in combination. All the cases with vivax malaria received primaquine prophylaxis. Only one case ended in fatality. 58 cases recovered completely. However, 3 cases were discharged against medical advice. Among the control group, in spite of being malaria negative, 40/62 patients received antimalarial treatment. All QBC positive and smear confirmed patients were included as cases.

DISCUSSION

Malaria is one of the serious parasitic diseases. In malaria endemic countries treatment is based on clinical diagnosis though its accuracy is limited [8]. The accurate diagnosis of malaria requires rapid, sensitive and specific tests that are available at affordable cost.

In the present study, total of 124 samples from 124 clinically suspected malaria cases were analyzed. The cases of fever

with QBC positive and QBC negative results were further studied for clinical signs and symptoms.

In the current study, *Plasmodium vivax* (64.5%) was the most prevalent species to cause infection. Infection by *P. falciparum* was seen in 16.1% of cases and mixed infection by both *P. vivax* and *P. falciparum* accounted for 19.35% cases. Other studies from this area have detected *P. vivax* in 25% of cases. *P. falciparum* and mixed infection by *P. vivax* and *P. falciparum* was noted in 29.2 % and 9.7% respectively in their study [6]. Our findings are similar to the one more study by Gurung et al., where *P. vivax* and *P. falciparum* were identified in 52.9 and 45.7 % of cases respectively [9].

The age group of 21-30 years comprising of 45.2% cases was most commonly affected followed by age group 11-20 (21%) years. Our results were comparable to study by Arif et al., in which 38% of cases were detected in the age group of 21-30 followed by 25% in 31-40 group [10]. A clear male preponderance of 82.3% against 17% in females is emerging in this study. Males spend a lot of time outdoors and in some societies men tend to sleep outside hence are at higher risk of exposure to mosquito bite. Other studies also have found higher incidence of malaria among males compared to females [10,11].

SDM College of Medical Sciences and Hospital being an important Tertiary Hospital in North Karnataka, patients from surrounding places come for medical treatment here. Highest number of cases 38.7% (24/62) belonged to the neighboring Koppal district while 30.6% cases were from the native Dharwad district followed by 11.4% in Haveri district.

A few signs and symptoms are referred to as cardinal features of malaria. Fever, chills, anaemia, splenomegaly, rigors and icterus are frequently associated with malaria [11]. Many or most of these signs and symptoms belong to the symptomatology of other infections too. The 124 cases included in the present study were grouped as cases of malaria (62) and cases of fever not due to malaria (62) based on QBC test.

In the present study, fever was seen in 100% of cases, chills in 88.7%, headache in 41.9% cases, rigors in 33.9%, pallor in 37.1%, icterus, splenomegaly and vomiting in 22.6%, hepatosplenomegaly in 17.7%, pain abdomen in 12.9%, hepatomegaly in 11.3% of QBC positive cases and altered sensorium was observed in 1.6% of cases. Among the QBC negative controls, fever was the predominant symptom followed by chills and rigors in 82.3% and 33.9% respectively. Headache was noted in 29%, pain abdomen in 24.2%, pallor in 19.4%, hepatomegaly in 11.3% patients. Splenomegaly and vomiting was found in 9.7% of controls. Hepatosplenomegaly, altered sensorium and icterus was seen only in 6.5%, 4.8% and 3.2% of control patients. Our study results are comparable to the study by Jelia et al., they reported fever in 100% cases followed by vomiting in 52%, headache in 34%, jaundice in 27%, pain abdomen in 07%, impaired consciousness in 04%. They also noted splenomegaly in 75% of patients followed by anaemia in 57%, icterus in 28%, hepatomegaly in 04 % and

hepatosplenomegaly in 19% of malaria cases [11]. Present study results also correlate to the study done at Tirupathi by Lepakshi et al., on vivax malaria where fever was noted in 100% of cases, headache in 48%, jaundice in 42%, vomiting in 38% and pain abdomen in 38%. Their study also showed splenomegaly in 56%, hepatomegaly in 52%, pallor and icterus in 48% and 42% of malaria cases [12].

The degree of association between the signs, symptoms and the final diagnosis of malaria can be studied by applying the statistics of concordance. The analysis would reveal how far the symptoms or signs in the study subjects can be compared between the cases (positive by QBC) and the matched controls (QBC negatives). The reproducibility of this association as a study of reliability of the degree of association was calculated by the Kappa Statistic [13].

The Kappa values derived for various signs and symptoms are presented in [Table/Fig-6]. The splenomegaly and icterus have highly significant association while pallor had a significant association with the diagnosis of malaria. Chills and rigors have been traditionally very strongly linked to malaria. However, the present study shows that these symptoms are non-significant indicators of malaria.

Clinical Feature	K-value	p-value
Splenomegaly	0.275	0.002 (Significant)
Icterus	0.24	0.001 (Significant)
Pallor	0.238	0.007 (Significant)
Headache	0.128	0.167 (Not significant)
Hepatomegaly	0.124	0.154 (Not significant)
Vomiting	0.115	0.201 (Not significant)
Pain abdomen	0.086	0.283 (Not significant)
Chills	0.081	0.213 (Not significant)
Rigors	0.066	0.473 (Not significant)
Altered sensorium	0.032	0.309 (Not significant)

[Table/Fig-6]: Concordance of symptoms/signs among cases and controls.

In the concordance study referred to above significance of pallor with diagnosis of malaria has been noted. Among the females included in the study 90.9% were anemic while 72.5% of males showed anaemia. National Family Health Survey by Government of India showed the prevalence of anaemia in general population in Karnataka among women and men aged 15-49 years as 44.8% and 18.2% respectively. Prevalence of anaemia in malaria affected individuals in the present study is well above the prevalence of anaemia (90.9% >44.8% in females and 72.5% >18.2% in males) as indicated by National Family Health Survey by Government of India [14]. Malaria is one of the common cause of anaemia in Indian population. Degree of anaemia corresponds to duration and severity of infection [7]. The correlation between leucocyte count and diagnosis of malaria didn't reveal any significant association.

As per the WHO definition, 95.1% in this study could be classified as thrombocytopenia [15]. The platelet count therefore is very strongly associated with malaria. In other clinical studies, thrombocytopenia was present in 89.13% [16]. Results were also comparable to study by Arif et al., where thrombocytopenia was observed in 79% of cases, among these mild, moderate and severe degree of thrombocytopenia were seen in 35.45%, 41.77% and 22.78% respectively [10].

In this part of the country both dengue and malaria are prevalent [17]. Cases presenting with fever and thrombocytopenia are often a diagnostic dilemma for the clinicians. Both the conditions are potentially dangerous, therefore, the value of laboratory diagnosis cannot be overemphasized. Laboratory tests having high sensitivity and specificity would avert disastrous outcome in morbid patients.

Total 58 cases recovered completely. Three cases were discharged against medical advice and one case ended fatally. The patient who succumbed to the infection was a 34-year-old, chronic alcoholic male and had vivax infection. It is essential to diagnose all the suspected cases of malaria accurately and treated adequately.

LIMITATION

In the present study, malaria cases were diagnosed based on the results of QBC, no molecular method was used. This study was conducted in a tertiary care hospital where the patients are referred after the initial symptomatic treatment at the peripheral centers which might have masked a few signs and symptoms among the patients included in the study.

CONCLUSION

Malaria is one of the leading causes of death in tropical and subtropical countries. *Plasmodium vivax* was the most common species to cause infection in this geographical area. Arriving at clinical diagnoses and differentiating the illness from other common tropical infections based on patients' signs and symptoms may be difficult. Splenomegaly, icterus and pallor are better predictors of malaria. Anaemia and thrombocytopenia are now commonly encountered complications with both *P. vivax* and *P. falciparum*. Therefore, high suspicion, precise clinical diagnosis and confirmation of diagnosis using laboratory tests are crucial in timely initiation of treatment with recommended antimalarial drugs to reduce morbidity and mortality from this infection. *Plasmodium vivax* infections must be paid careful attention as they also are potentially fatal.

REFERENCES

- [1] Singh G, Urhekar AD, Maheshwari U, Sharma S, Raksha. Prevalence of malaria in a tertiary care hospital in Navi Mumbai, India. *J Bacteriol and Parasitol*. 2015;6:221.
- [2] Singh R, Kumar S, Rana SK, Thakur B, Singh SP. A comparative study of clinical profiles of Vivax and Falciparum malaria in children at a tertiary care centre in Uttarakhand. *J Clin Diagn Res*. 2013;7(10): 2234-37.
- [3] Magnitude of the problem. [Internet] Available from <http://www.nvbdc.gov.in/malaria3.html>. [last updated 2014].
- [4] WHO, 2008, Malaria Rapid Diagnostic Test Performance Summary results of WHO Malaria RDT Product Testing: Rounds 1-3 (2008-2011) Geneva. 2011.
- [5] Sarkar SS, and Dasgupta D. Comparative study of modified quantitative buffy coat and two rapid tests in comparison with peripheral blood smear in malaria diagnosis in Mumbai, India. *Journal of Parasitology Research*. vol. 2014, Article ID 194651, 7 pages, 2014.
- [6] Salmani MP, Preeti BM, Peerapur BV. Comparative study of peripheral blood smear, QBC and antigen detection in malaria diagnosis. *J. Clin Diagn Res*. 2011;5:967-69.
- [7] Garcia LS. *Diagnostic Medical Parasitology* : 5th ed. (ASM Press Washington, DC) 2007: pages 142-189.
- [8] Nkrumah B, Agyekum A, Acquah SE, May J, Tannich E, Brattig N, et al. Comparison of the novel partec rapid malaria test to the conventional giemsa stain and the gold standard real-time PCR. *J Clin Microbiol*. 2010;48(8):2925-28.
- [9] Gurung B, Bairy I, Jagadishchandra, Manohar C. Evaluation of Falcivax against quantitative buffy coat (QBC) for the diagnosis of malaria. *International Journal of Collaborative Research on Internal Medicine & Public Health*. 2010;2(5):132-40.
- [10] Arif M, Jelia S, Meena SR, Meena S, Jain P, Ajmera D, et al. A study of thrombocytopenia in malaria and its prognostic significance. *Int J Res Med Sci*. 2016; 4(6): 2373-78.
- [11] Jelia S, Meena S, Meena SR, Arif M, Jain P, Ajmera D, et al. A study of clinical profile and complication of malaria in a tertiary care centre in South-eastern region of Rajasthan, India. *Int J Adv Med*. 2016;3(3):614-20.
- [12] Lepakshi G, Padmaja N, Sandeep. Study of clinical profile of patients with plasmodium vivax malaria. *Journal of Evidence Based Medicine & Healthcare*. 2015;2:3380-85.
- [13] Rosner B. *Fundamentals of Biostatistics*. 7th ed. BROOKS/COLE Cengage learning; 2011:404-09.
- [14] National family health survey 2015-16, Karnataka Fact sheet released by Ministry of Health and Family welfare. Government of India. Downloaded from website rchiips.org/NFHS/pdf/NFHS4/KA_FactSheet.
- [15] Maina RN, Walsh D, Gaddy C, Hongo G, Waitumbi J, Otieno L. Impact of *Plasmodium falciparum* infection on haematological parameters in children living in Western Kenya *Malaria Journal* 2010;9(Suppl 3):S4.
- [16] Nadkar MY, Huchche AM, Singh R, Pazare AR Clinical Profile of Severe *Plasmodium vivax* Malaria in a Tertiary Care Centre in Mumbai from June 2010-January 2011. *JAPI* 2012;60:11-13.
- [17] Islam MN, ZulKifle M, Sherwani AMK, Ghosh SK, Tiwari S. Prevalence of malaria, dengue, and chikungunya significantly associated with mosquito breeding sites. *The Journal of IMA*. 2011;43(2):58-67.

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