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Research Article

ASSOCIATION OF CLINICAL FEATURES WITH *P. FALCIPARUM* POSITIVITY IN A TERTIARY CARE HOSPITAL OF NORTH INDIA

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Abstract: Classical malaria due to any of the species present with typical clinical paroxysm which consists of three stages, an initial chill stage, febrile stage and the third stage or sweating stage. Infections with *P. falciparum* can however deteriorate very quickly to more severe presentations, now defined under the broad terms of severe malaria. The study aims to guide the clinicians in tertiary care settings to correlate the clinical sign and symptoms prior to starting the anti-malarial on clinical suspicion alone so as to minimize the emerging problem of anti-malarial drugs overdose and resistance. The present study was conducted in Postgraduate Department of Microbiology, CSMMU (now King George's Medical University), Lucknow over a period of one year from August 2008 to August 2009. The study was carried out as a hospital based observational study. Blood samples were collected in EDTA vials. Each sample was examined by Giemsa stained thick and thin smears. Chi square test, Inter-observer Differences ("Kappa"), Logistic Regression, Multivariate Analysis were used to predict the outcomes. A total of 107 samples from clinically suspected cases of malaria were enrolled for the study from the medicine and pediatric wards of Gandhi Memorial and Associated Hospitals, CSMMU, Lucknow, out of which 42 samples were positive for *P.falciparum* infection. A statistically significant ($p < 0.001$) association of Fever with continuous pattern was the most common clinical features while diarrhoea and decreased renal out-put were the least common. Hypotension/collapse and haemoglobuminuria were found to be absent in all the cases. Among the observed clinical features only temperature >102°F, vomiting, altered consciousness, jaundice and anaemia seem to be significantly associated with the outcome Microscopic Positivity. The clinical criteria for malaria diagnosis though very strict and less sensitive, has very high specificity, *i.e.* clinical criteria has the capability to rule out the negative cases effectively.

Keywords: Malaria, *P. falciparum*, Giemsa method.

INTRODUCTION

Malaria is a protozoan disease transmitted by the bite of infected female anopheles mosquitoes. It is the most important of all tropical diseases in terms of morbidity and mortality. More than two billion people (36% of world population) are exposed to the risk of contracting malaria[1,2]. Each year, malaria directly causes nearly one million deaths and about 500 million clinical cases, of which 2 to 3 million constitute severe and complicated malaria [3,4].

The classic malarial paroxysms, in which fever spikes, chills, and rigors occur at regular intervals, are relatively unusual and suggest infection with *P. vivax* or *P. ovale*. The fever is irregular at first (that of falciparum malaria may never become regular); the temperature of non-immune individuals and children often rises above 40°C in conjunction with tachycardia and sometimes delirium. In some instances, a prominence of headache, chest pain, abdominal pain, arthralgia, myalgia, or diarrhea may suggest another diagnosis. Headache may be severe in malaria. myalgia may be prominent, it is not usually as severe as in dengue fever, and the muscles are not tender. Nausea, vomiting, and orthostatic hypotension are common. In non-immune individuals with acute malaria, the spleen takes several days to become palpable, but splenic enlargement is found in a high proportion of otherwise healthy individuals in malaria-endemic areas and reflects repeated infections. Slight enlargement of the liver is also common, particularly among young children. Mild jaundice is common among adults; it may develop in patients with otherwise uncomplicated falciparum malaria and usually resolves over 1–3 weeks (Harrison's Principle of Internal Medicine, 17 edition, volume I, Chapter 203, pg 1283)[5].

The accepted "gold standard" laboratory procedure for diagnosis of malaria is the examination of Giemsa stained thick blood smears under the microscope. In tertiary setting like large teaching hospital in areas endemic for malaria, unique problems are encountered in diagnosis of malaria. As patients reaching such centers are critically ill, the clinician start presumptive anti-malarial therapy, sometimes even

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before dispatch of any laboratory samples. Patients may have possibly received partial presumptive therapy even before reaching the center, hence may be smear negative. This study was planned to study the association of clinical sign and symptoms with *P. falciparum* positivity in a tertiary care hospital in north India.

MATERIALS AND METHODS

Study site and time

The present study was conducted in Postgraduate Department of Microbiology, Chhatarapati Shahuji Maharaj Medical University (now King George's Medical University), Lucknow over a period of one year from August 2008 to August 2009. CSMMU is a tertiary care referral center catering to the needs of Lucknow and other adjoining districts of U.P. Cases are usually referred to the center when they do not benefit from the treatment at the local health care facility or when they require critical care.

Study design

The study was carried out as a hospital based observational study.

Inclusion criteria

Patient with an initial differential diagnosis of malaria in which:

1. Request for malaria parasite test by the treating clinician is made.
2. Administration of empirical anti-malarial therapy by the clinician, 24 hours prior to or after ordering the malaria parasite test.

Note: Cases were enrolled consecutively except for Sundays and Holidays.

Study population

The study population comprised of clinically suspected cases of malaria. All the patients enrolled in the study had malaria as one of their differential diagnosis. Cases were recruited from Medicine and Pediatrics Wards of Gandhi Memorial and Associated Hospitals, CSMMU, Lucknow.

Variables

The study variables were documented in each case as per the set protocol in the screening form and included fever along with jaundice, anaemia, altered consciousness, splenomegaly, pulmonary edema, convulsions and decreased renal output to decide about the severity of malaria cases in our hospital setting.

Microscopy for malaria parasite

Blood samples were collected in EDTA vials. Each sample was examined by Giemsa stained thick and thin smears. (*Shute GT*, 1988)[6]

Statistical analysis

The statistical analysis was done using SPSS (Statistical Package for Social Sciences) Version 15.0 statistical Analysis Software. The values were represented in Number (%).

The following Statistical formulas were used:

1. **Chi square test:**

$$\chi^2 = \frac{\Sigma(O-E)^2}{E}$$

Where O = Observed frequency
E = Expected frequency

2. **Inter-observer Differences ("Kappa"):**

$$\text{Kappa, K} = \frac{p_o - p_e}{1 - p_e}$$

where, p_o = proportion of agreement observed
p_e = proportion of agreement expected

3. **Logistic Regression:** Logistic regression makes no assumption about the distribution of the independent variables. They do not have to be normally distributed, linearly related or of equal variance within each group. The relationship between the predictor and response variables is not a linear function in logistic regression, instead, the logistic regression function is used, which is the logit transformation of :

$$\theta = \frac{e^{(\alpha + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k)}}{1 + e^{(\alpha + \beta_1 x_1 + \beta_2 x_2 + \cdots + \beta_k x_k)}}$$

Where = the constant of the equation and, = the coefficient of the predictor variables.

4. **Level of significance:** "p" is level of significance

p > 0.05	Not significant
p <0.05	Significant
p <0.01	Highly significant
p <0.001	Very highly significant

RESULT

Table 1/Fig1: Ward wise Distribution of Study Population (n=107)

S. No.	Ward	No. of cases	Percentage
1.	Medicine	82	76.6
2.	Pediatrics	25	23.4

Table 2/Fig2: Month wise Distribution of study population (n=107)

S. No.	Month	No. of cases	Percentage
1.	June	6	5.6
2.	July	16	15.0
3.	August	50	46.7
4.	September	20	18.7
5.	October	14	13.1
6.	November	1	0.9
	Total	107	100.0

†Maximum number of cases were enrolled in the month of August (46.7%).

Table 3: Area wise Distribution of study population (n=107)

S. No.	Area	No. of cases	Percentage
1.	Rural	57	53.27%
2.	Urban	50	46.73%

†Almost equal number of cases enrolled in the study belonged to Rural (53.27%) and urban area (46.73%).

Table 4: Age wise Distribution of study population (n=107).

Age group	No. of cases	Percentage
0-5	12	11.2
6-10	9	8.4
11-20	21	19.6
21-30	20	18.7
31-40	18	16.8
41-50	14	13.1
51-60	7	6.5
>60	6	5.6

†Maximum number of patients were in the age range 11-50 years (68.2%). In the age group >60 years, there were only 5.6% subjects.

Table 5: Gender wise Distribution of Study Population

Gender	No.	%
Male	66	61.7
Female	41	38.3

†There were 66 (61.7%) males and 41 (38.3%) females. Male: Female ratio of cases was approximately 1.5:1.

Table 6: Smear Positivity for malaria Parasite by Giemsa Stained Thick and Thin Smear (all are positive cases were of *P.falciparum* infection).

S. No.	Smear	Thick Smear		Thin Smear	
		No.	%	No.	%
1.	Positive	42	39.3	42	39.3
2.	Negative	65	60.7	65	60.7

χ^2=1.00; p<0.001

†Giemsa stained thick and thin smear revealed 42 (39.3%) specimen to be positive and 65 (60.7%) specimen to be negative. There was complete concordance between thick and thin smear results.

Table 7: Presence of Anaemia on the basis malaria positivity by microscopy

S.No.	Microscopy Status	Total anaemia positive	Males (n=66)	Females (n=41)
1.	Positive (n=42)	33	18 (54.54%)	15 (45.45%)
2.	Negative (n=65)	35	21 (60%)	14 (21.53%)

†Percentage calculated columnwise

†Incidence of anaemia was found to be more common amongst males both amongst microscopy positive as well as microscopy negative patients.

Table 7: Distribution of Various Clinical Features in Clinically Suspected Cases of Malaria

S. No.	Clinical Feature	No. of cases	Percentage
1.	Fever	107	100
2.	Fever ≥102°F	84	78.5
3.	Fever >10d	63	58.9
4.	Recurrent pattern	92	86.0
5.	Chills	20	18.7
6.	Vomiting	56	52.3
7.	Diarrhoea	9	8.4
8.	Abdominal pain	33	30.8
9.	Headache	64	59.8
10.	Altered consciousness	45	42.1
11.	Convulsions	8	7.5
12.	Hypotension/collapse	0	0
13.	Decreased RO	4	3.7
14.	Pulmonary edema	5	4.6
15.	Hepatomegaly	53	49.53
16.	Anaemia	68	63.55
17.	Haemoglobminuria	0	0
18.	Splenomegaly	34	31.77
19.	Jaundice	49	45.79

†Fever with continuous pattern was the most common clinical features while diarrhoea and decreased RO were the least common. Hypotension / collapse and haemoglobuminuria were found to be absent in all the cases.

Table 8: Association of clinical features with malaria positivity in study population

S. No.	Clinical Features	Total No. (n=107)	Malaria positive (n=42)	Malaria negative (n=65)	χ^2	p
1.	Fever	107	42	65	–	–
2.	Continuous pattern of fever	92	40	52	4.915	0.027
3.	Fever <10 days	63	27	36	0.835	0.361
4.	Vomiting	51	26	25	5.621	0.018
5.	Diarrhoea	9	1	8	3.342	0.068
6.	Abdominal pain	33	17	16	2.833	0.092
7.	Headache	64	24	40	0.304	0.581
8.	Altered consciousness	45	24	21	6.145	0.013
9.	Convulsions	8	1	7	2.595	0.107

†Among the above only continuous pattern of fever, vomiting and altered consciousness seemed to be significantly associated with the diagnosis of malaria.

Table 9: Association of clinical signs with malaria positivity in study population

S. No.	Clinical Signs	Total No. (n=107)	Malaria positive (n=42)	Malaria negative (n=65)	χ^2	p
1.	Hypotension	0	0	0	–	–
2.	Pulmonary edema	5	0	5	3.500	0.061
3.	Hepatomegaly	53	20	33	0.158	0.691
4.	Anaemia	68	33	35	6.290	0.012
5.	Haemoglobuinuria	0	0	0	–	–
6.	Splenomegaly	34	13	21	0.040	0.841
7.	Jaundice	49	26	23	6.879	0.009

†Except for anaemia and jaundice no other variable had a significant association with the diagnosis.

Table 10(A): Multivariate Analysis to predict outcome (Malaria positivity) Iteration: 1Variables in the Equation

Step 1 (a)	B	S.E.	Wald	df	Sig.	Exp (B)
Temp>102°C	2.024	.843	5.765	1	**0.016**	7.571
Pattern	1.488	.945	2.479	1	0.115	4.426
Chills	-.409	.765	.286	1	0.593	0.664
Vomiting	1.536	.647	5.645	1	**0.018**	4.646
Diarrhoea	-2.102	1.465	2.058	1	0.151	0.122
AbdominalPain	.086	.618	.019	1	0.889	1.090
Headache	-.010	.599	.000	1	0.987	0.990
AlteredCons	1.386	.646	4.612	1	**0.032**	4.000
Convulsions	-.502	1.326	.143	1	0.705	0.605
Decrease R.O	3.063	1.802	2.890	1	0.089	21.394
Pulmonary Edema	-20.198	16175.972	.000	1	0.999	0.000
Hepatomegaly	-.817	.624	1.715	1	0.190	0.442
Jaundice	1.679	.682	6.058	1	**0.014**	5.361
Splenomegaly	-.733	.640	1.310	1	0.252	0.481
Anaemia	2.400	.781	9.435	1	**0.002**	11.024
Constant	-6.006	1.564	14.742	1	0.000	0.002

†a Variable(s) entered: Temp>102, Pattern, Chills, Vomiting, Diarrhoea, Abdominal Pain, Headache, Altered Cons, Convulsions, Decrease R.O, Pulmonary Edema, Hepatomegaly, Jaundice, Splenomegaly, Anaemia.

†Only temperature >102°F, vomiting, altered consciousness, jaundice and anaemia seem to be significantly associated with the outcome Microscopic Positivity.

Second Iteration

Table 10 (B): Variables in the Equation

Step 1(a)	B	S.E.	Wald	df	Sig.	Exp(B)
Temp	1.869	0.727	6.612	1	**0.010**	6.479
Vomiting	0.983	0.508	3.741	1	0.053	2.673
AlteredCons	1.495	0.537	7.752	1	**0.005**	4.460
Jaundice	1.301	0.508	6.571	1	**0.010**	3.673
Anaemia	1.870	0.582	10.310	1	**0.001**	6.490
Constant	-4.972	1.044	22.684	1	0.000	0.007

†a Variable (s) entered on step 1: Temp, Vomiting, AlteredCons, Jaundice, Anaemia.

†Second iteration showed rejection of vomiting.

Third iteration

Table 10 (C): Variables in the Equation

Step 1(a)	B	S.E.	Wald	df	Sig.	Exp(B)
Temp	1.875	0.719	6.797	1	0.009	6.520
AlteredCons	1.553	0.525	8.754	1	0.003	4.724
Jaundice	1.411	0.495	8.129	1	0.004	4.099
Anaemia	1.642	0.550	8.919	1	0.003	5.164
Constant	-4.418	0.957	21.328	1	0.000	0.012

a Variable (s) entered on step 1: Temp, Altered Cons, Jaundice, Anaemia.

†Temperature above 102°F, altered consciousness, jaundice and anaemia were found to be significantly associated.

Table 11: Diagnostic Efficacy of Clinical Criteria (Temperature 102°F, altered consciousness, jaundice and anaemia) in clinically suspected patients of malaria

Clinical Criteria	Microscopic Diagnosis		Total
	+	–	
+	9 (a)	1 (b)	10
–	33 (c)	64 (d)	97
Total	42	65	107

χ^2=98.994 (df=1); p<0.001

Sensitivity = a/(a+c) = 9/42=21.42 %

Specificity= d/(b+d) = 64/65= 98.46%

Positive predictive value = a/(a+b) = 9/10 = 90%

Negative predictive value = d/(c+d) = 64/97 = 65.97%

†The clinical criteria, though very strict and less sensitive has high specificity, *i.e.* it has the capability to rule out the negative cases effectively.

DISCUSSION

Correlation of clinical features and use of anti-malarials to the positivity of study population on microscopy

The clinical features present in the study population are described in table-7. This predominance of *P. falciparum* is likely due to the setting of the study, rather than the distribution of malaria parasite species in the local geographical area. Patients who come to tertiary care centers are likely to be suffering from severe malaria which is caused by *P. falciparum*. Similar results had previously been suggested by Molyneux *et al.*, (1993)[7]. In this study, among 86 (80%) cases with clinically suspected malaria who were given presumptive antimalarial therapy, only 42 (39%) cases were found to be positive by microscopy and they were all given antimalarial therapy. The remaining 42 of 65 cases, that were negative on microscopy, were also given antimalarial therapy. This reflects enormous pressure on part of clinicians to start antimalarial, based on clinical suspicion alone, even when initial laboratory reports are not available.

Among the different clinical criteria included in the study, continuous pattern of fever (p value 0.027), altered consciousness (p value 0.013), anaemia (p value 0.012) and jaundice (p value 0.009) seemed to be significantly associated with the positive diagnosis of malaria by microscopy. All the above mentioned clinical features if present together are significantly associated with malaria positivity by microscopy. When the sensitivity of all these clinical criteria were compared with the microscopy positive result, it was found to be very low (21.43%) but the specificity (98.46%) and PPV (90%) were high. Thus we conclude the discussion by the inference that clinical criteria for malaria diagnosis though very strict and less sensitive, has very high specificity, *i.e.* clinical criteria for the diagnosis of *P. falciparum* malaria has the capability to rule out the negative cases effectively.

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