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Original Research Article

Analysis of correlation between the parasite density and clinical profile of malaria

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ABSTRACT

Introduction: Malaria, an infectious disease caused by protozoans of the genus Plasmodium, continues to be a serious global health problem. Typically, a higher parasite count is associated with a more severe infection and increased mortality. Delay in diagnosis and treatment also contributes to the mortality. In India, transmission of malaria is low and seasonal.Due to this unstable endemicity of the disease, we hypothesized that in our patients with malaria, morbidity and mortality will occur at lower parasitaemia levels compared to highly endemic areas. Therefore, we conducted this study at our tertiary care centre to find out correlation between parasite density and clinical profile of malaria.

Materials and Methods: This observational, hospital based, cross- sectional study was carried out to find the correlation between parasite density and clinical profile of malaria in department of Pediatrics, Maharana Bhupal Government hospital, Udaipur for one year (April 21 to March 2022) duration.

Results: A total of 96 children aged 1 month to 18 years of age were enrolled. The most common presenting feature was fever (100%) followed by nausea and vomiting (68.8%) followed by headache (43.7%), jaundice (31.3%) and pain abdomen (29.2%). 60.4% patients had parasite density of less than 50,000 followed by 20.8% with below 1 lakh parasite density, 14.6% with density between 1 to 2 lakhs and remaining with higher parasite density. Maximum parasite density (more than 1 lakh/μl) was observed among P. falciparum patients, followed by mixed infection (1-2 lakhs/μl) patients. Correlation of clinical profile and parasite density revealed that as the parasite density increased there was increased in number of patients with headache, impaired consciousness, convulsions, and oliguria.

Conclusion: High parasite density was associated with severe clinical illness and deranged laboratory parameters. As parasite density is very sensitive index, preparation of good quality peripheral blood film and proper assessment (parasite density) can help to assess the disease severity and outcome. High parasitaemia can be prevented by general measures in the form of proper education, good sanitation, and good awareness about the use of anti-larval activity.

1. Introduction

Malaria, an infectious disease caused by protozoans of the genus Plasmodium, continues to be a serious global health problem, especially in the tropics and subtropics. 241 million people became ill from malaria in 2020 across 87 countries. 627,000 people died from malaria in 2020. Approximately 2.48 million malaria cases are reported annually from South Asia, of which 75% cases are contributed by India alone.[1,2]

The WHO has updated the criteria for identifying severe malaria, and many factors are utilized for defining severity.[3] Cases of severe malaria can be caused by Plasmodium falciparum or Plasmodium vivax, although traditionally, the

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progression to severe and lethal forms has been attributed mainly to infections by the former species.[4]

There have been evidences of relationship between parasite density and variation in the prognosis of patients with severe malaria as a function of the level of disease transmission.[5] High levels of parasitemia constitute a potential risk factor for complications and death from this cause. In non-immune children and adults, in areas of unstable endemicity, peripheral parasitaemia of 4% or more carries an increased risk of death and is considered as a sign of severe malaria. While in areas of stable endemicity, parasite density 20% is considered as severe malaria. Parasite density can serve as a good prognostic marker combined with other established factors. Owing to its low cost and effectiveness, the parasite index should be evaluated in all malaria patients.[6,7]

In India, transmission of malaria is low and seasonal.[8] Due to this unstable endemicity of the disease, we hypothesized that in our patients with malaria, morbidity and mortality will occur at lower parasitaemia levels compared to highly endemic areas. Therefore, we conducted this study at our tertiary care centre to find out correlation between parasite density and clinical profile of malaria and to study the complications and outcome of malaria in relation with parasite density.

2. Materials and Methods

This observational, hospital based, cross sectional study was done in Department of Pediatrics, Maharana Bhupal Government hospital, Udaipur for a duration of one year (April 21 to March 2022). Inclusion criteria were children aged 1 month to 18 years, clinically suspected patients of malaria and patients with positive peripheral blood film and rapid diagnostic test positive for malaria. Exclusion criteria included peripheral blood smear or rapid diagnostic test negative for malaria parasite, any other co-infection and coexisting morbid conditions like diabetes mellitus, chronic renal disease and chronic liver disease.

Approval for study was obtained from the institutional ethical committee. An informed consent was taken from all the patient's parents/guardians.

2.1. Methodology

A detailed history and clinical examination was performed for all patients. Investigations were done in all patients, including a complete blood count, peripheral blood smear (thin and thick), blood sugar, serum bilirubin, aspartate aminotransferase, alanine aminotransferase, urea, creatinine, serum proteins, serum electrolytes, chest X-ray, and arterial blood gas, if needed.

Blood samples were collected at the time of admission and both the thick and thin smears were prepared separately for each patient. The blood films were air dried and thin blood films fixed with methanol. Both thick and thin blood films were stained with Leishman´s solution. Leishman´s stained thick blood films routinely examined for the detection of malarial parasites. The initial thick smear declared negative only if no malarial parasites were seen after the examination of 100x/1.25 oil immersion high power fields. At least 100 oil immersion fields in thick smear and 200 oil immersion fields in thin smear were examined before reporting the smear as negative for malaria parasite. If blood smear was negative for malaria parasite despite strong clinical suspicion, repeat smears were taken.

After the detection of malarial parasites, thin smears were examined to identify the parasite species. After the confirmation of the species, parasite density was estimated also from the thin blood smears and the level of parasitaemia was expressed as percentage (%) of erythrocytes infected with malarial parasites.

2.2. Calculations

2.2.1. Parasite count and parasite density

In a thick smear, number of parasites was counted till 200 white blood cells are observed. Number of parasites present per μl of blood was calculated from the following formula:

Total leucocyte count/μL $\times$ [No. of parasite] / 200

Usually, total leukocyte count is taken as 8000/ μl and the number of parasites is multiplied by 40 to get the result. However, if accurate leukocyte count is known, then a better estimate of parasite density is obtained. To obtain percent parasitaemia, figure of number of parasites amongst 200 white blood cells is divided by 1250.

In a thin smear, number of parasites amongst 1000 red cells is counted and reported as a percentage. Number of parasites in 1μl of blood can be calculated if red cell count in millions/μl is known; if it is not known, then it can be arbitrarily taken as 5 million/ μl.

Number of parasites in 1μl of blood = Red cell count in million/cmm $\times$ parasite percentage

The parasite density was estimated daily for 3 days. Mean parasite density was calculated and used to estimate correlations with clinical manifestations, complications, and outcome in malaria patients.

All the patients received treatment according to the WHO protocol in malaria endemic areas:- Artesunate 2.4mg/kg intravascular followed by 2.4mg/kg at 12hr and 24 hours and continue injection once daily if necessary. If no response / improvement with artesunate after 48 hours, Quinine 20mg/kg was infused during 4 hours followed by 10 maintenance 10mg/kg during 2-8 hours every 8 hours. Complete monitoring of the patient was done during the hospital stay.

2.2.2. Statistical analysis

Data was entered in excel sheet. Continuous data was summarised in form of mean and standard deviation.

Difference in mean of two groups was analysed using student's' test. Continuous data was expressed in form of proportions and difference in proportions was analysed using chi-square test. The level of significance was kept 95% for all statistical analysis.

3. Results

A total of 96 patients aged 1 month to 18 years of age were enrolled in the study. Maximum number of patients were in the age group of 10-18 years (32;33.33%) followed by 6-10 years (25; 26.04%) and 3-6 years (18;18.75%). Youngest child was 2- month- old and oldest was 18 years old. Male to female ratio was 0.95:1. The most common presenting feature was fever which was present in all patients (96; 100%). Nausea and vomiting were the second most complaint (66; 68.8%) followed by headache (42;43.7%), jaundice (30;31.3%) and pain abdomen (28;29.2%). Convulsion was the least common presentation in 5 (5.2%) patients (Table 1).

The most common species of malarial parasite was P. falciparum seen in 67 (69.79%) patients followed by P. vivax infection (22; 22.92%). Maximum number of patients (58; 60.42%) had parasite count <50000/μl, followed by 50000-100000/μl in 20 (20.83%) and 100000-200000/μl in14 (14.58%) patients. Parasite count between 200000-250000/μl and >250000/μl was found in 2 patients each and that too in p. falciparum infection (Figure 1). Amongst the all patients, maximum (44; 45.83%) had fever of more than 5 days duration. Twenty- seven (28.13%) patients had fever of 1-3 days duration followed by 3-5 days (20; 20.83%) and less than 24 hours (5; 5.21%). Maximum patients of P. falciparum presented after 5 days of onset on fever while the patients with p. vivex and mixed infection were admitted within 1-3 days of fever. On correlation of duration of fever and parasite density in children with malaria (Figure 2), 100% patients who were admitted with fever of more than 5 days duration had maximum parasite density (100000 - >250000/μl).

Correlation of headache and parasite density revealed that as the parasite density increased there was increased in number of patients with headache and this difference was statistically significant (Table 2).

Correlation of jaundice with parasite density showed that as the parasite density increased, number of patients with jaundice also increased from 20.7% to 40%. Similar percentage (50%) of patients fall in the parasite density of 1 lakh to 2 lakhs and 2 lakhs to 2.5 lakhs group. Further increase of patients was seen in range of more than 2.5 lakhs parasite density (100%). This difference was statistically significant. No direct relation was observed between parasite density and pain abdomen (p value >0.05) in the study population.

Maximum percentage of patients (50%) presented with impaired consciousness had higher parasite density compared to patients with low parasite density. However, the difference was statistically insignificant (p value >0.05). With the increasing parasite density upto 2.5 lakhs/ μl, increased number of patients were reported to have oliguria. The difference was statistically significant (p value 0.00).

Patients with moderate hepatosplenomegaly and severe anemia had high parasite density and this was statistically significant (p value 0.01). High parasite density was well correlated with leucopenia, thrombocytopenia, raised liver and renal enzymes.

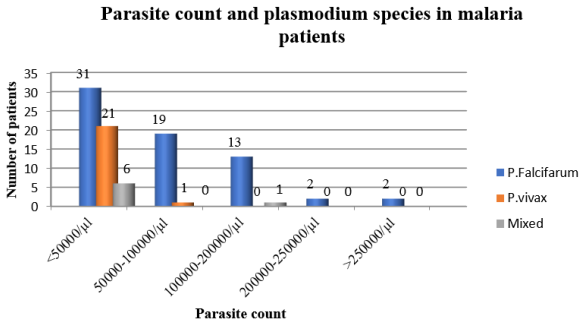

Fig. 1: Parasite count and plasmodium species in malaria patients.

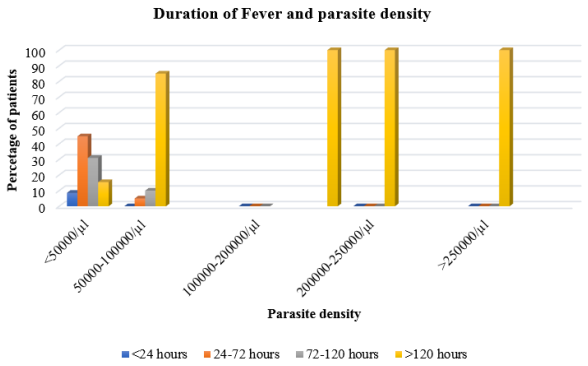

Fig. 2: Duration of fever and parasite density.

4. Discussion

In this hospital based cross-sectional observational study, a total of 96 children aged 1 month to 18 years of age were enrolled. One third of the patients were adolescents, followed by 6 to 10 years old (26%), pre -school age group (18.7%) and toddlers (13.5%). Mean age was 7.96 years with SD 4.76. The mean (SD) age of the patients was 4.4 (2.0) years in the study by Waller et al.[9]

Almost equal number of male and female children were enrolled in our study with male to female ratio of 0.95:1. In our study approximately same proportion of male and female patients were noted in P. vivax (male-45.5%, female- 54.5%) and P. falciparum infections

Table 1: Clinical profile of patient with malaria

S. No.	Clinical profile	Number of cases (%)
1	Fever	96 (100)
2	Nausea and vomiting	66 (68.8)
3	Headache	42 (43.7)
4	Jaundice	30 (31.3)
5	Pain abdomen	28 (29.2)
6	Cough	20 (20.8)
7	Oliguria	19 (19.8)
8	Impaired consciousness	18 (18.7)
9	Convulsion	5 (5.2)

Table 2: Correlation of headache with parasite density

Parasite count	Headache		Total (%)
	Yes	No	
<50000/μl	16(27.6%)	42(72.4%)	58(100)
50000-100000/μl	12(60%)	8(40%)	20(100)
100000-200000/μl	11(73.3%)	4(26.7%)	15(100)
200000-250000/μl	2(100%)	0(0%)	2(100)
>250000/μl	1(100%)	0(0%)	1(100)
Total	42(43.7%)	54(56.3%)	96(100%)
P-value 0.001			

Table 3: Hepatomegaly and parasite density

Parasite count	Hepatomegaly				Total
	No hepatomegaly	Mild (<3 cm)	Moderate (4-7 cm)	Severe (>7 cm)	
<50000/μl	13(22.4%)	39(67.2%)	6(10.3%)	0(0.0%)	58(100)
50000-100000/μl	2(10%)	17(85.0%)	1(5.0%)	0(0.0%)	20(100)
100000-200000/μl	0(0.0%)	10(71.4%)	4(28.5%)	0(0.0%)	14(100)
200000-250000/μl	0(0.0%)	1(50%)	0(0%)	1(50%)	2(100)
>250000/μl	0	0(0%)	2(100%)	0	2(100)
Total	15(15.9%)	67(69.8%)	13(13.5%)	1(1.0%)	96(100%)
P-Value 0.01					

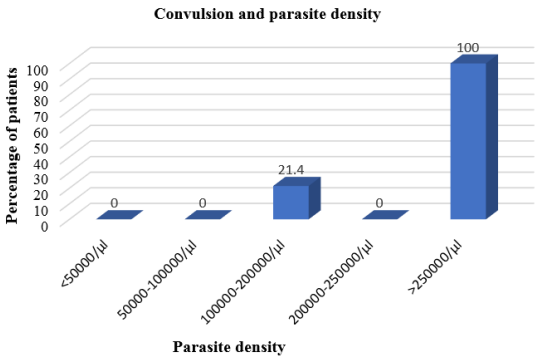

Fig. 3: Convulsion and parasite density in study population.

(male- 49.3%, female- 50.7%) group. In the study by Kochar et al,[10] male/female child ratio was 70.6% versus 29.4%, the proportion of female children was higher in P. vivax infections (33% [34/103]) compared with P. falciparum infections (27.1% [50/185]; odds ratio [OR] = 1.3 [95% confidence interval (CI) = 0.79–2.24], P = 0.352). This predominance of female children with severe P. vivax infection was more apparent from 0–5 year age group (57.1% [4/7]) and reaching 80% (12/15) in 5–10 year age group and 100% (11/11) in > 10 year age group.

Maximum patients in our study were diagnosed with plasmodium falciparum (69.7%), followed by P. Vivax (22.9 %) and mixed (7.2%) infections. In the study by Kochar et al,[10] the proportion of P. falciparum, P. vivax and mixed infection was 61.01%, 33.99%, and 4.95%, respectively. The age-stratified composition of different species of malaria was P. vivax monoinfection 33.9% (103/303) (children aged 0–5 years 42.3% [41/97]; in 5–10 years 30.1% [44/146]; > 10 years 30% [18/60]) compared with P. falciparum monoinfection 61.01% (185/303) (children aged 0–5 years 51.5% [50/97]; in 5–10 years 65.1% [95/146]; > 10 years 66.7% [40/60]) and mixed (Pf + Pv) infection 4.95% (15/303) (children aged 0–5 years 6.2%

[6/97]; in 5–10 years 4.8% [7/146]; > 10 years 3.3% [2/60]).

In the index study, 60.4% patients had parasite density of less than 50,000 followed by 20.8% with below 1 lakh parasite density, 14.6% with density between 1 to 2 lakhs and remaining with higher parasite density. In the study by Mangal et al,[11] 68% of patients had a parasite density of < 5%, 18% had a parasite density of 5 to 10% and 14% had a parasite density of >10%.

Most common presenting complaint in our study was fever (100%), followed by nausea and vomiting (68.8%), headache (43.7%), jaundice (31.3%), pain abdomen (29.2%), cough (20.8%) and oliguria (19.8%). In the findings by Mangal et al,[11] fever was the most common symptom and was present in all patients. Nausea and vomiting (66%), headache (62%), jaundice (22%), impaired consciousness (17%), oliguria or anuria (13%), and bleeding (4%) were also observed. Convulsions were not present in any case. Shaikh et al[12] also reported fever in all patients, rigor in 96% of patients, and vomiting and headache in 62% of patients. Ali et al[13] also observed fever in 100% of cases. Murthy et al[14] reported fever with chills and rigor (98.10%), altered sensorium (48.10%), algid malaria (18.35%), and jaundice (27.12%). Fever was most common clinical feature (100%) as compare to other symptoms like convulsion (78%), unconsciousness (67%), altered sensorium (33%), pain abdomen (7%) and breathlessness (7%) in study by Khandelwal S.[15] Fever (100%), seizures (66%), vomiting (62%), and abdominal pain (47%) were also common presenting features in study by Waller et al.[9]

Maximum parasite density (more than 1 lakh/μl) was observed among P. falciparum patients, followed by mixed infection (1-2 lakhs/μl) patients. Kochar et al[10] observed parasite density between 7,600–60,000/mm^3 in P. vivax malaria.

Amongst the all patients, maximum (44; 45.83%) had fever of more than 5 days duration. Twenty- seven (28.13%) patients had fever of 1-3 days duration followed by 3-5 days (20; 20.83%) and less than 24 hours (5; 5.21%). Maximum patients of P. falciparum presented after 5 days of onset on fever while the patients with p. vivex and mixed infection were admitted within 1- day of fever. In the study by Kochar et al[10] mean duration of fever was 5.4 days with SD 3.1 days. Fever prevalence among children was 9.4% (766/8,816) in the study by Mabunda et al.[16] In their study, the prevalence of malaria infections associated with fever peaked among children in the less than twelve months age group and thereafter decreased rapidly with increasing age ($p < 0.001$). High parasite densities were significantly associated with fever ($p < 0.04$). In our study also correlation of duration of fever and parasite density showed that 100% patients who were admitted with fever of more than 5 days duration had maximum parasite density (100000 - >250000/μl). Study by Khandelwal S[15] and Darraj MA[17] revealed that high grade fever was associated with high parasitic index.

Correlation of headache and parasite density revealed that as the parasite density increased there was increased in number of patients with headache and this difference was statistically significant (p-value 0.001). Pro-inflammatory cytokines such as TNF-α and IL-6 are believed to play an important role in the pathogenesis of headaches in malaria.[18]

Maximum percentage of patients (50%) presented with impaired consciousness had higher parasite density compared to that patient with lower parasite density. Similar findings were observed by Khandelwal S[15] and Mangal et al,[11] and this difference was statistically significant in their studies.

With the increasing parasite density upto 2.5 lakhs/ μl, increased number of patients were reported to have oliguria. The difference was statistically significant (p value 0.00). Mangal et al[11] also showed similar observation.

As the parasite density increased, increased number of patients were reported to have convulsions (Figure 3). This difference was statistically significant (p value 0.00). Khandelwal S[15] also reported that 67.9 % patients of convulsion had high parasitic index.

In our study severe anaemia was seen in 51 patients (53.1%) and higher parasite density (2 lakhs and above) was present in these children. Moderate anaemia was present in 35 (36.4%) patients and parasite density was <50000/μl in more than half of these patients. This correlation was statistically significant (p value 0.01). Severe anemia (hemoglobin < 5 g/dL) was present in 81% (64/79), 75.4% (49/65), and 33.3% (2/6) children having P. falciparum, P. vivax, and mixed infections, respectively, as per Kochar et al.[10] In the studies by Mangal et al[11] and Shaikh et al[12] anemia was found in 58% of cases each. Anemia results from accelerated RBC removal by the spleen, obligatory RBC destruction during parasite schizogony, and ineffective erythropoiesis. Enlargements of the liver and spleen result from the inflammatory response to plasmodia and are more severe in cases of P. falciparum infection.

On laboratory parameters, as the parasite density increased number of patients with hepatomegaly (Table 3), raised SGOT and SGPT, serum creatinine and urea also increased. Similar findings were reported in other studies[11,19] also. Hepatic dysfunction was present in 44.3% (35/79), 26.2% (17/65), and 16.7% (1/6) children having P. falciparum, P. vivax, and mixed infections, respectively, as per Kochar et al.[10] They reported renal dysfunction among 30.4% (24/79) and 15.4% (10/65) children having P. falciparum and P. vivax infections, respectively. The mean level of blood urea and serum creatinine was 193.9 ± 47.153 mg/dL and 3.680 ± 0.653 mg/dL, respectively.

In the index study, as the parasite density increased, more number of patients were reported to have thrombocytopenia. Other studies[20,21] also reported similar observation.

Abnormal bleeding was present in the findings by Kochar et al[10] in 17.7% (14/79), 10.8% (7/65), and 16.7% (1/6) children having P. falciparum, P. vivax, and mixed infections, respectively. Thrombocytopenia is thought to be caused by increased splenic sequestration, immune-mediated destruction, and shortened platelet survival. The degree of thrombocytopenia is associated with the severity of falciparum malaria.

5. Conclusion

High parasite density was associated with severe clinical illness and deranged laboratory parameters. As parasite density is very sensitive index, preparation of good quality peripheral blood film and proper assessment (parasite density) can help to assess the disease severity and outcome. High parasitaemia can be prevented by general measures in the form of proper education, good sanitation, and good awareness about the use of anti-larval activity.

6. Source of Funding

None.

7. Conflicts of interest

There are no conflicts of interest.

8. Acknowledgements

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