ORIGINAL ARTICLE

Parasite Density and The Spectrum of Clinical Illness in Falciparum Malaria

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ABSTRACT

Objective: To determine the impact of percentage parasitemia and clinical features on morbidity and mortality in patients with *P. falciparum* malaria.

Study Design: Case series.

Place and Duration of Study: Department of Medicine, Medical Unit II, Jinnah Postgraduate Medical Centre, Karachi, Pakistan from May to November 2005.

Patients and Methods: Seventy-six adult patients of smear positive *P. falciparum* malaria were selected for the study. Parasite density was estimated on thin blood film and expressed as percentage of red blood cells parasitized. Patients were divided into three groups on the basis of parasite density. The data was analyzed on SPSS version 12. Results were expressed as percentages, mean and standard deviations. P-value <0.05 was taken as significant.

Results: Data of 76 study patients who fulfilled the inclusion criteria was analyzed on the basis of parasite density. Thirty-one (40.79%) patients had parasite density < 5%, 22 (28.95%) had parasite densities between 5% and 10% and 23(30.26%) patients had parasite density >10%. Comparative analysis of the groups showed that pallor, impaired consciousness, jaundice or malarial hepatitis, thrombocytopenia, acute renal failure, DIC, and mortality were all strongly associated with the density of *Plasmodium falciparum* malaria (p=0.001). Parasite density was not related to age, gender and hepatosplenomegaly.

Conclusion: High parasite density was associated with severe clinical illness, complications and mortality. Parasite counts of > 5% may be considered as hyperparasitaemia in this population of the world.

Key words: *Parasite density. P. falciparum malaria. Complications. Mortality.*

INTRODUCTION

Infection with *Plasmodium falciparum* (PF) is more serious than with other malarial species, because of frequency of severe and fatal complications associated with it. This lethal parasite can be the basis to cerebral malaria, acute renal failure, acute malarial hepatitis, hypoglycaemia, hyperpyrexia, non-cardiogenic pulmonary oedema, adult respiratory distress syndrome, adrenal insufficiency-like syndrome, hyperparasitaemia, Blackwater fever, cardiac arrhythmias and gastrointestinal syndromes like secretory diarrhea.[1-3]

Plasmodium falciparum infects erythrocytes of any age with the potential of development of high-grade parasitaemia.[4] Due to the rapid multiplication of this parasite, the parasite count can increase upto 20-fold over a period of 48 hours without treatment.[5] In the clinical setting, the level of parasitaemia is useful as one of the criteria in defining "severe *P. falciparum* malaria" and to monitor the effect of anti-malarial therapy.[6]

It is well documented that in *P. falciparum* malaria, a direct correlation exists between an individual's asexual erythrocytic-stage parasite density at the time of presentation to a health care provider and the severity of clinical disease.[7] Patients with high parasite count have more severe and complicated course.[7,8] Mortality is also correlated with the degree of parasitaemia. Patients with the highest parasite densities have the highest case fatality rates.[8,9] Exchange transfusions may be beneficial if the parasite count is above 10%, as it might be expected to reduce the parasitaemia more rapidly than optimal chemotherapy alone.[10]

The relationship between the parasite density and severity of illness in *P. falciparum* malaria is not straight forward. This relation is different in different populations and age groups. 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 a sign of severe malaria.[11] While in areas of stable endemicity, parasite density ≥ 20% is considered

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severe malaria.[11] In Pakistan, transmission of malaria is low and seasonal; cases of *P. falciparum* malaria are usually seen in the second half of year after July-August monsoon rains. Due to this unstable endemicity of the disease, we hypothesized that in our patients with falciprum malaria, morbidity and mortality will occur at lower parasitaemia levels compared to highly endemic areas of Africa, where most of the studies are reported from.

The objective of the study was to demonstrate the impact of increasing parasite density on severity of disease pattern and adverse outcome in patients with *P. falciparum* malaria.

PATIENTS AND METHODS

This open prospective case series study was conducted in the Department of Medicine, Medical Unit II, Jinnah Postgraduate Medical Centre (JPMC), Karachi, Pakistan over a period of 2.5 years between May 2003 and November 2005. Our unit is one of the three medical units in the department of medicine with 53 in-patient beds. It receives patients every third day.

This study included 76 adult patients of both sexes and different ages who presented to us with history of fever and were confirmed to have *P. falciparum* malaria, by demonstration of asexual forms of PF in their peripheral blood smears. Patients were categorized into three groups depending upon parasite density. Group A with < 5% parasite density, Group B with parasite density between 5% to 10% and Group C with >10% parasite density. Patients who had negative peripheral smear for PF, or had mixed plasmodium infection, and those who had evidence of any other disease or condition contributing towards the complications and affecting the outcome of *P. falciparum* malaria, were excluded from the study. Majority of the patients were very sick and admitted through the accident and emergency department.

Details of history and clinical assessment were noted in all patients on a pre-tested questionnaire. World Health Organization (WHO) criteria were used to assess the severity and to describe the systemic complications of *P. falciparum* malaria.[11] All patients underwent a set of investigations such as complete blood count, general peripheral blood film for evidence of haemolysis, thick and thin blood films for malarial parasite, blood sugar, ECG and X-ray chest.

Blood samples were collected at the time of admission and sent to the Kidney Centre Laboratory where both thick and thin smears were prepared separately for each patient. The blood films were air dried and thin blood films were fixed with methanol. Both thick and thin blood films were stained with Leishman´s solution for 15 minutes and were then washed gently in tap water.

Leishman´s stained thick blood films were routinely examined for the detection of malarial parasites. The initial thick smear was declared negative only if no malarial parasites were seen after the examination of 100x/1.25 oil immersion high power fields by the expert microscopist of the Kidney Centre.

After the detection of malarial parasites, thin smears were used to identify the parasite species. After the confirmation of PF 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.

In this method, 1,000 erythrocytes were examined and the number of parasitized Red Blood Cells (RBCs) among these were noted and % parasitaemia was then calculated by dividing the number of parasitized erythrocytes by the total number of RBCs indexed and multiplied by 100.[13]

$$\%\text{ age Parasitaemia} = \frac{\text{Number of parasitized RBCs} \times 100}{\text{Total RBCs counted (1000)}}$$

Liver function tests included, serum ALT, AST, bilirubin, total proteins, albumin and prothrombin time. Renal profile included urea, and creatinine and electrolytes. Ultrasonography of abdomen, prothrombin time, D-dimer, bleeding time, arterial blood gases, cerebrospinal fluid examination and blood cultures were done when indicated.

All patients received specific treatment in the form of IV quinine or I/M artemether using the standard regimen.[13] Whereas, specific complications were treated according to the WHO protocol.[13] Complete monitoring of the patient was done during the hospital stay. Daily follow-up was carried out for the early detection of complications and the therapeutic response to drugs. All patients were followed in hospital till recovery or death.

To assess the impact of increasing parasite density on clinical disease of *P. falciparum* malaria, the patients were divided into three groups, Group A with <5%, Group B with 5-10% and Group C with > 10%. A database on the basis of pre-filled proformas was developed on SPSS version12. The quantitative variables were described by their mean ± SD values. Analysis of variance (Fisher's-test, one-way ANOVA) was performed to compare the mean difference among three groups of patients. The categorical data presented by their percentages and difference in proportions was compared by chi-square test of proportions. Difference in mean values between two variables strata were evaluated with the Student's t-test. P-value of < 0.05 was taken as significant for all statistical analysis.

RESULTS

A total of 76 patients of *P. falciparum* malaria who fulfilled the inclusion criteria were enrolled in the study. There

were 60 (78.9%) males and 16 (21.1%) females who ranged in age between 13-70 years (mean ± SD= 32 ± 13.89 years). Most of the patients were young without evidence of co-morbidities.

Fever was the leading symptom. All patients were febrile at the time of admission. Anaemia or pallor (72.4%), Thrombocytopenia (76.3%), hepatosplenomegaly (59.2% and 55.3%), malarial hepatitis (47.36%), impaired consciousness (46.05%), and acute renal failure (40.78%) were the most common presenting abnormalities.

The impact of increasing parasite density on clinical disease pattern on the three groups of patients with *P. falciparum* malaria is shown in Table II.

No statistically significant difference was detected between the mean parasite densities of male and female patients 8.08 ± 9.13% vs. 5.96 ± 6.31% respectively (p=0.38 by t-test). Although the mean age was slightly higher in patients with parasite density more than 10%, it did not vary significantly amongst the three groups of patients with incremental parasite density P=0.181.

Although fever was the major presenting symptom, there was no association between parasite density and presence of fever. The fever did not increase with the increase in the level of parasite density (p=0.23 Table I and II).

Moreover, symptomatic disease occurred even at low parasitaemia levels. Case summaries' descriptive analysis showed that among the 76 febrile patients, 13 (17.1%) had parasite count <0.08%.

There was a positive correlation between duration of illness and parasite count. The patients with prolonged duration of illness (>7 days) were more likely to have higher degree of parasitaemia levels (p=0.0001) compared to short duration (< 7 days) (Table II).

Impaired consciousness was found to be the indicator of severity of *P. falciparum* malaria. Assessment on Glasgow Coma Scale (GCS) score showed a significant association between the increasing level of parasite density and impaired consciousness and hence the low GCS score.

Impaired consciousness was present in 16.13% of patients with parasite counts of <5% with GCS score of 14 ± 2.25 and 73.91% of patients with parasitaemias >10 % (mean ± SD) had a GCS score of 11.48 ± 3.10.

Presence of hepatosplenomegaly was not associated with high parasite count. Liver and spleen were enlarged and palpable in majority of the patients irrespective of their parasite density (p=0.076 and 0.391 respectively Table I). Jaundice (malarial hepatitis) was strongly associated with parasite density and increased with increasing level of parasite density.

Malarial hepatitis was diagnosed in 12.90% of patients with parasite count <5%, as compared to 86.96% of patients with parasite density >10%. This showed the significant impact of high parasite count on the hepatic involvement (p=0.0001). A positive correlation between the serum bilirubin and high parasite count was

Table I: Association between parasite density and clinical characteristics of 76 studied patients.

Clinical parameter	Group-A Parasite density < 5%	Group-B Parasite density 5 - 10 %	Group-C Parasite density > 10 %	p-value
	no =31 (40.79%)	no = 22 (28.95%)	no = 23 (30.26%)	
Gender;				
Male	23 (74.2)	16 (72.7)	21 (91.3)	0.218
Female	8 (25.8)	6 (27.3)	2 (8.7)	
Fever	31(40.79)	22 (28.95)	23 (30.26)	0.23
Impaired consciousness	05 (16.12)	13 (59.09)	17 (69.56)	0.001
Anaemia or pallor	17 (54.83)	18 (81.82)	20 (86.96)	0.017
Hepatomegaly	15 (48.39)	12 (54.54)	18 (78.26)	0.076
Splenomegaly	19 (61.19)	13 (59.09)	10 (43.47)	0.391
Thrombocytopenia	18 (58.06)	18 (81.82)	22 (95.65)	0.004
Malarial hepatitis.	04 (12.90)	12 (54.54)	20 (86.95)	0.0001
Oliguria or anuria	02 (6.45)	06 (27.27)	13 (56.52)	0.000
Renal failure	03 (9.68)	10 (45.45)	18 (78.26)	0.0001
Cerebral malaria	03 (9.68)	07 (31.82)	07 (30.43)	0.088
D.I.C.	0 (0)	02 (9.09)	05 (21.74)	0.024
Expired	02 (6.45)	06 (27.27)	14 (60.87)	0.0001

Table II: Comparision between parasite density and laboratory parameters of 76 studied patients.

Laboratory parameters (mean ± SD)	Group-A (no.=31) Parasite density < 5%	Group-B (no.=22) Parasite density 5 -10%	Group-C (no.=23) Parasite density >10%	p-value
Age	30.06 ±14	30.04 ± 11.04	36.47 ± 15.61	0.181
Duration of illness (days)	4.65 ± 1.77	6.18 ± 3.01	7.52 ± 2.89	0.0001
Temperature in F^0	101.22 ± 1.82	101.40 ± 1.59	100.61 ± 1.44	0.230
Haemoglobin (g/dl)	9.70 ± 2.07	7.52 ± 2.62	6.94 ± 2.26	0.0001
WBC count ($x10^9$/L)	6.25 ± 3.04	6.96 ± 3.61	10.28 ± 4.36	0.0001
Platelet count ($x10^9$/L)	108.98 ± 56.69	74.86 ± 69.91	39.22 ± 30.02	0.0001
Total bilirubin (mg/dl)	1.79 ± 1.25	5.59 ± 7.95	15.12 ± 10.73	0.0001
Serum ALT (U/L)	39.06 ± 20.32	45.77 ± 20.80	54.13 ± 27.66	0.092
Serum AST(U/L)	42.77 ± 21.05	65.95 ± 67	78.78 ± 65.47	0.028
Serum total proteins (mg/dl)	6.75 ± 0.48	6.58 ± 0.63	6.38 ± 0.52	0.054
Serum albumin (mg/dl)	3.37 ± 0.49	3.16 ± 0.58	2.65 ± 0.43	0.0001
Prothrombin time (seconds)	14.48 ± 1.09	14.77 ± 1.23	16.69 ± 3.58	0.001
Blood urea (mg/dl)	49.45 ± 39.55	148.68 ± 111.71	246.87 ± 133.35	0.000
Serum creatinine (mg/dl)	1.58 ± 1.16	4.05 ± 3.23	7.02 ± 4.32	0.0001
Glasgow coma scale score	14 ± 2.25	12.41 ± 3.06	11.48 ± 3.10	0.005
Parasite density (%)	1.93 ± 1.39	7 ± 1.93	15.93 ± 11.35	0.000

observed. The bilirubin level increased from 1.79 ± 1.25 mg/dl to 15.12 ± 10.73 mg/dl in patients with parasite density <5% and parasitaemia levels >10 % (p=0.0001).

Surprisingly the rise in serum alanine aminotransferase (ALT) was mild to moderate and was not significantly related to the increase in parasite density. Patients with parasite count >10% had mean levels of ALT 54.13 ± 27.66 IU/L compared to 39.06 ± 20.32 IU/L in patients with parasite density <5 % (p=0.092). The mean ± SD prothrombin time increased from 14.48 ± 1.09 (seconds) to 16.69 ± 3.58 (seconds) and serum albumin level decreased from 3.37 ± 0.49 mg/dl to 2.65 ± 0.43 mg/dl as parasite count increased from < 5% to >10% respectively (p=0.0001, Table II). Reduced albumin level and high prothrombin time were indicative of hepatic dysfunction in patients with high parasite count.

Anaemia was noted in 72.4% of patients. On further analysis of subgroups of patients; on the basis of parasite density, we found that 86.96% of the patients with parasite count >10% were anaemic, at the time of admission compared to 54.83% of patients with parasite density <5% (p=0.017, Table I). A strong inverse relationship between haemoglobin level and parasite density was observed. The mean ± SD haemoglobin levels decreased from 9.70 ± 2.07 g/dl to 6.94 ± 2.26 g/dl as parasite count increased from <5% to >10% respectively (p=0.0001 Table II).

Overall thrombocytopenia was seen in 76% of patients. Further analysis revealed that there was parallel increase in the incidence of thrombocytopenia with increasing *P.falciparum* density in all three groups. Thrombocytopenia was present in 95.65% of patients with parasite count >10% and 58.06% of patients with parasite count <5 % (p = 0.004 Table I). Moreover, a trend was observed in which the mean ± SD values of platelet counts were decreased with increasing density of *P. falciparum*. Patients in Group-C had significantly low (p=0.0001) platelet counts of 39.22 ± 30.02 $x10^9$ /L compared to Group-B and Group-A patients who had platelet counts of 74.86 ± 69.91 x 10^9/L and 108.98 ± 56.69 x 10^9/L respectively (Table II).

Presence of high leukocyte count was significantly more common in patients with parasite count > 10%. The mean ± SD white blood cell (WBC) count of patients with parasite density >10% was 10.28 ± 4.36x10^9/L compared to 6.25 ± 3.04 x 10^9/L in patients with parasite density < 5% (p=0.0001, Table II).

Acute Renal Failure (ARF) was significantly associated with high parasite density. Majority of the patients with ARF were oliguric. Comparison of the three groups revealed a strong linear dose response relationship in which the risk of oliguric renal failure increased with increasing level of parasite density. 78.26 % of patients had evidence of renal failure when parasite density was > 10% compared to 45.45% when parasite count was between 5% to 10% and this risk was further reduced to 12.90% when parasite density was <5% (p=0.0001).

It was observed that with increasing density of *P. falciparum*, magnitude of derangement of renal function parameters increased. The mean ± SD values of blood urea and serum creatinine increased from 49.45 ± 39.55 mg/dl and 1.58 ± 1.16 mg/dl respectively to 246.87 ± 133.35 mg/dl and 7.02 ± 4.32 mg/dl respectively when parasite density increased from <5% to >10% (p=0.0001).

It was observed that patients with *P. falciparum* malaria started to develop complications at parasite count ≥ 5% and severity of complications increased when parasite count was >10%. Among 76 studied patients, 49 (64.73%) fulfilled the WHO criteria[5] of severe malaria with 41(83.76%) of these had parasite density >5% (chi-square p=0.0001). Of the 76 patients, 22 (28.95%) succumbed to the disease, while 54 (71.05%) survived.

Mortality reached to 60.87% in patients with parasite density >10% compared to 6.45% in patients with <5% parasite density and 27.27% in patients with parasite densities between 5% and 10% (p=0.0001, Table I). Moreover, on further analysis by student t-test, it was observed that the parasite density (mean± SD) value was significantly higher (14.77 ± 12.42 %) in patients who expired compared to those who survived (4.73 ± 3.77 %) p=0.0001. Patients with high parasite count had multiple complications of *P. falciparum* malaria which were responsible for death in that individual.

DISCUSSION

The clinical course of *P. falciparum* malaria varies according to the level of endemicity. In highly endemic areas of Africa, where transmission of malaria is high, clinical illness normally reaches a peak in children under 05 years of age and declines substantially later.[15] In these areas, children are reported to have considerable parasitaemia without febrile illness and parasite count > 5% is well tolerated especially in adult population without leading to complications.[16] On the other hand in those areas where endemicity or transmission of malaria is low, like Pakistan, cases of severe *P. falciparum* malaria occur in adults, adolescent as well as in older children having symptomatic illness at lower levels of parasitaemia. This is because of the delayed development of immunity to infection and hence protection from the disease.[16,17]

Clinical illness compatible with malaria in our subjects was associated with lower levels of parasitaemia. 19.5% of patients developed symptoms of malarial disease at parasite counts of <0.08%. Moreover, parasite density of > 5% was associated with development of complications and severity of these complications increased at parasite counts of >10%. Symptomatic infection in the presence of low parasite load and occurrence of complications at parasite counts of > 5%

suggest that there was little immunity against the *Plasmodium falciparum* infection in our patients. Occurrence of severe *P. falciparum* malaria at parasite counts of >5% and delayed development of clinical immunity to *Plasmodium falciparum* has been described in many studies[18,19] in adult population especially in areas where infection is patchy and seasonal.

The typical clinical manifestations of malaria i.e. fever was recorded in all 76 patients. The presence of fever and degree of elevation of body temperature were not related to the severity of parasitaemia and all patients were febrile at any level of parasitaemia. Peterson and others also found no correlation between history of fever and elevated body temperature and parasitaemia levels among 121 Liberian adult patients with *P. falciparum* malaria.[20] WHO report of severe malaria 2000 also reiterates this point.[13]

The impairment of consciousness in patients with high parasite count was predicted as a sign of severe infection. Piarroux R *et al.* have documented in their study conducted in Marseilles, France that neurological signs such as impaired consciousness is an effective and simple way to diagnose potentially severe cases of *Plasmodium falciparum*.[21] Lalloo *et al.* have also reported similar results in their study conducted in Papua New Ginea.[22]

Haematological abnormalities are considered a hallmark of malaria and reported to be most pronounced in *P. falciparum* infection. Although the pathogenesis of anaemia in malaria is extremely complex and incompletely understood, it is commonly seen in patients with *P. falciparum* malaria.[23] It may develop rapidly, taking a serious turn in *P. falciparum* infection due to heavy parasiteamia. At higher levels of parasitaemia, excessive haemolysis of parasitized RBCs may lead to anaemia.[24]

Anaemia observed in 72.4% of patients was found associated with high parasite count. An inverse relationship between parasite densities and admission haemoglobin levels were observed.

Cases of *Plasmodium falciparum* with low platelet counts have been documented in other populations of semi-immune and non-immune patients.[25] Thrombocytopenia was seen in majority of our patients. It was observed that at high parasitaemia, the platelets were significantly low. The trend of decreasing platelet counts with increasing levels of parasitaemia observed in this study has been previously noted for *Plasmodium falciparum* by other authors.[25,26]

The presence of jaundice in *P. falciparum* malaria indicates a more severe illness with a higher incidence of complications and poor prognosis. There is a strong association between jaundice and high parasite count.[27] Anand *et al.* has also reported similar results from india.[28] In our study jaundice was noted at high parasite count. Risk of having jaundice in the study subjects increased from 12.90% to 86.95% when parasite count increased from <5% to 10%.

P. falciparum malaria is one of the most common causes of ARF in adults. It is seen in those areas of the tropics where transmission of the malaria is unstable and where symptomatic disease occurs at all ages.[12] One of the factors causing acute renal failure in patients with *Plasmodium falciparum* is high parasite count.[12] We have reported the occurrence of ARF in this study. Most of the patients who developed ARF had high parasite density. Moreover, majority of the patients with ARF also had jaundice and hepatomegaly, especially at higher levels of parasite density. The association of jaundice and ARF in this study is comparable with the past studies. Nacher *et al.* reported from Thailand that jaundice and hepatomegaly were significantly associated with acute renal failure but not with cerebral malaria.[29] In Vietnamese adults, 63% of those with ARF were jaundiced compared to 20% of those without renal failure.[19]

Plasmodium falciparum is the most dangerous form of malaria with a mortality exceeding 20% depending on the degree of parasitaemia and the development of complications.[4] High parasitaemia even without complications can lead to high mortality. Mortality can reach upto 50% in patients with parasitaemia greater than 10% in areas of low transmission.[12]

In our study, *Plasmodium falciparum* was associated with substantial mortality that parallels the degree of parasitaemia and development of complications. The substantial mortality rate observed in the present study in those patients who had high parasite counts is in agreement with other studies conducted in areas with similar pattern of malaria transmission.[19,22]

WHO guidelines recommend exchange blood transfusion if more than 10% of the red blood cells are parasitized in the presence of severe disease, especially cerebral malaria, acute renal failure, ARDS, jaundice and severe anaemia. A recent meta-analysis demons-trated that a greater benefit from adjunct exchange transfusion was seen in patients of Asian origin with partial background immunity against malaria.[31]

Exchange transfusion was not performed in any of our patients. If adjunct exchange transfusion would have been performed perhaps the mortality would have been reduced further.

CONCLUSION

The study demonstrated that severe *P. falciparum* malaria is an important problem in this part of the world. Unlike

in Sub-Saharan Africa, it occurs in adults at parasite density ≥ 5% and there is delayed development of immunity. High parasite density was associated with severe clinical illness, complications and mortality.

Recommendations:

1. The presence of pallor, jaundice, altered sensorium, low platelet and renal failure at the time of presentation in patients with *Plasmodium falciparum* malaria should be considered as surrogate markers of high parasite density.
2. Exchange blood transfusions may be considered as an early therapeutic intervention in patients who present with this pattern of illness in order to reduce mortality.
3. We further recommend large sample size studies to reduce the mortality.

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