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Comparative study of clinical presentation and hematological indices in hospitalized sickle cell patients with severe *Plasmodium falciparum* malaria

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ABSTRACT

Background: Sickle-cell-gene has a high frequency in malaria endemic regions. In India, though the prevalence of both sickle-cell-gene and malaria are high, no study has been carried out. This study aims to find out the possible differences in hematological and clinical parameters in severe *falciparum* malaria with respect to sickle cell genotypes.
Methods: Five hundred fourteen adults with severe *falciparum* malaria hospitalized in Department of Medicine, Veer Surendra Sai Institute of Medical Sciences and Research, Burla, between August, 2010 to December, 2014 were included and categorized on the basis of sickle cell genotypes. The hematological parameters were compared by one-way-analysis-of-variance and incidence of sub-phenotypes of severe malaria was compared by $\chi 2$ test across the groups.
Results: Patients with sickle cell anemia (HbSS) and severe *falciparum* malaria had lower hemoglobin level compared to patients with normal β-globin genotype (HbAA) and sickle cell trait (HbAS). Most of the hematological parameters were homogeneous in patients with HbAA and HbAS and different from patients with HbSS. Incidence of acute renal failure was low ($\chi 2$, 9.91; p, 0.002) and jaundice was high ($\chi 2$, 5.20; p, 0.022) in patients with HbSS. No clinical difference was observed in patients with HbAA and HbAS. The mortality was low ($\chi 2$, 4.33; p, 0.037) and high ($\chi 2$, 10.48; p, 0.001) in patients with HbAS and HbSS respectively compared to patients with HbAA.
Conclusion: Though sickle-cell-gene protects against *falciparum* infections, the hematological parameters and sub-phenotypes of severe malaria remain unchanged when the infection progresses to a severe form in patients with HbAA and HbAS. Presence of hemolytic anemia in patients with HbSS shows diverse hematological and clinical phenotypes as compared to others. High mortality in patients with HbSS emphasizes the need for a better preventive approach to save valuable lives.

Introduction

After decades of implementing various control strategies, *Plasmodium falciparum* (*P. falciparum*) malaria continues to be a major public health problem in the Indian subcontinent. According to the National Vector Borne Disease Control Programme [1], there were 0.85 million total reported cases of malarial infection (including 0.54 million *P. falciparum* infection) and 316 deaths. It has been reported that, the number of deaths due to malaria is 8.75 times higher in subjects ≥5 years compared to children <5 years

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[2]. This difference in mortality calls for a shifting of malaria control strategies towards adults rather than focusing only on women and children [2]. The severity of *P. falciparum* malaria ranges from asymptomatic parasitemia to severe disease manifestation and death [3]. In severe malaria, patients present with varied clinical signs and symptoms depending on their age, geographical co-ordinates, and influence of the host's as well as parasite's genetic factors [4].

Sickle cell gene is found to be protective against severe *P. falciparum* malaria and has attained high frequency in malaria endemic regions due to its selection pressure [5,6]. Most of the studies on the association of sickle cell gene and malaria have been undertaken in Africa. In India, the incidence of both *P. falciparum* malaria and sickle cell gene are high. In a recent cross-sectional study in a malaria endemic region, the prevalence of sickle allele was found to be 13.1% with an allelic frequency of 0.08% [7]. It has been found that individuals with sickle cell gene are suffering from severe *P. falciparum* malaria, in spite of the protective effect. There has been no study describing the association of sickle cell gene and *P. falciparum* malaria in India. Malaria is a major public health problem of Odisha state and the state is considered as a hyperendemic region with low perennial transmission with a seasonal peak from July to October [8]. The state contributes to 37.0% of malaria positive cases, 50% of *P. falciparum* cases and 18.0% of malaria related deaths in India [1]. This study aims to find out possible variations in the clinical and hematological parameters in severe *P. falciparum* malaria in presence of sickle cell gene.

Methods

This prospective cohort study was undertaken in Sickle Cell Clinic and Molecular Biology Laboratory, Department of Medicine, Veer Surendra Sai Institute of Medical Sciences and Research, Burla, Odisha, India. This tertiary care hospital caters to a population of about 12 million residing in western Odisha and eastern Chhattisgarh. The state of Odisha is situated along the Bay of Bengal and it extends from 17.78° N to 22.73° N latitude to 81.37° E to 87.53° E longitude. The study site is situated on the geographical co-ordinates of 21.50° N and 83.87° E.

Recruitment of cases

Patients aged 15–65 years with clinically suspected malarial infections, hospitalized in the Department of Medicine, Veer Surendra Sai Institute of Medical Science and Research, Burla, between August, 2010 to December, 2014 were included in the study. Severity of *P. falciparum* malaria was defined as per WHO criteria 2010 [9]. The most severe complication, cerebral malaria (CM) was defined as patients with any presentation like altered sensorium, convulsion or Glasgow Coma Score (GCS) of ≤10. Other complications like severe malaria anemia (SMA) (hemoglobin, <5 g/dl), acute renal failure (ARF) (serum creatinine >3 mg/dl), jaundice (serum bilirubin >3 mg/dl), hepatic dysfunction (alanine transaminase (ALT)/aspartate transaminase (AST) > 120 U/L), respiratory distress, hemoglobinuria (dark red or black colored urine positive for hemoglobin) and shock (systolic BP of <80 mm Hg) were considered. Written informed consent was obtained from all cases. This study was approved by the institutional ethical committee of Veer Surendra Sai Institute of Medical Science and Research, Burla.

Exclusion criteria

The following cases were excluded from the study (a) subjects co-infected with other *Plasmodium* species; (b) children <15 years of age; (c) subjects having associated chronic disease like tuberculosis, chronic renal failure, cirrhosis of liver and autoimmune diseases like systemic lupus erythematosus and rheumatoid arthritis; (d) patients with dengue fever; (e) pregnant women; (f) patients positive for hepatitis virus infection; (g) subjects who refused consent.

Hematological analysis

All the cases were subjected to either immune chromatography test (SD, Bio Standard Diagnostics India) or Quantitative Buffy Coat (QBC) (BD, Becton Dickinson Diagnostics) followed by single step polymerase chain reaction for confirmation of *Plasmodium* species [10]. All the cases were screened for sickle cell gene by sickling slide test. Those found positive were subjected to alkaline agarose gel Hb electrophoresis (pH 8.6) and high performance liquid chromatography (Biorad Variant-II using β-thalassemia short program). Complete blood count and biochemical parameters such as serum bilirubin, alanine transaminase, aspartate transaminase, creatinine, urea, sodium, potassium, glucose were carried out for all patients.

Data analysis

Statistical analysis was done using SPSS 16.0 and a p value < 0.05 was considered statistically significant. Comparison of means of various parameters between the groups was calculated by one way analysis of variance using Duncan Multiple Range Test (DMRT). Pearson $\chi 2$ tests were used for incidence of various clinical symptoms with respect to sickle cell genotype.

Results

Of the 588 blood samples collected with suspected severe *P. falciparum* infection, 514 were finally included in the study. The mean age of the patients was 34.0 ± 13.0 years (range, 15–65 years) with 69.0% (355/514) being males. Majority of the patients (55.6%; 286/514) belonged to 15–34 years of age. The commonest complication of severe *P. falciparum* malaria observed was cerebral malaria (34.2%) followed by acute renal failure (32.3%), jaundice (29.0%), breathing difficulties (22.4%), hepatopathy (20.8%), severe malarial anemia (11.9%), shock (6.0%) and hypoglycemia (4.3%). The clinical severity of the patients was represented with the presence of either single or multiple clinical sub-phenotypes. Thirty four (6.6%) patients died. Screening for sickle cell gene resulted in 402 (78.2%), 66 (12.85%) and 46 (8.95%) patients with normal β-globin genotype (HbAA), sickle cell trait (HbAS) and homozygous sickle cell anemia (HbSS) respectively. The mean age of patients were 34.9 ± 13.3, 34.8 ± 12.5 and 25.4 ± 8.8 years in patients with HbAA, HbAS and HbSS respectively (p, 0.093). The male to female ratio was found to be 2.62:1, 1.36:1 and 1.42:1 in patients with HbAA, HbAS and HbSS respectively. The demographic features and clinical presentations are enlisted in Table 1.

The Duncan Multiple Range Test for comparison of complete blood count data among the three genotypes of sickle cell gene showed there was a significantly decreased value of hemoglobin, RBC and hematocrit in patients with HbSS compared to patients with HbAS and HbAA. However, WBC and platelet counts were comparatively higher in patients with HbAS and HbSS although in normal range. MCV was low in patients with HbAS compared to both HbAA and HbSS. MCH and MCHC were comparable in all the three groups of patients. All the biochemical parameters were comparable in the three groups except serum urea, creatinine and LDH. Serum urea and creatinine were found to be higher in both HbAA and HbAS compared to patients with HbSS, whereas the level of LDH was significantly higher in patients with HbSS, compared to HbAA and HbAS. Duncan Multiple Range Test showed the majority of parameters were comparable in patients with HbAA and HbAS but was different in patients with HbSS (Table 2).

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