Association of ABO blood group with severe falciparum malaria in adults: case control study and meta-analysis

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Abstract

Background: Erythrocyte-associated antigenic polymorphisms or their absence have perhaps evolved in the human population to protect against malarial infection. Studies in various populations consistently demonstrate that blood group 'O' confers resistance against severe falciparum infection. In India, Odisha state has one of the highest incidences of Plasmodium falciparum infection and contributes to the highest number of deaths by falciparum malaria. This study aims to evaluate the relationship between ABO blood group and severe malaria in an adult population at the tertiary care centre in Odisha.

Methods: A total of 353 P. falciparum infected subjects and 174 healthy controls were screened for ABO blood group. Falciparum-infected individuals were categorized as severe malaria and uncomplicated malaria. Severe malaria was further clinically phenotyped into cerebral malaria, non-cerebral severe malaria and multi-organ dysfunction. A meta-analysis was performed to assess the role of ABO blood group in severe malaria.

Results: Frequency of blood group 'B' was significantly higher in patients with severe malaria compared to the uncomplicated cases (P < 0.0001; OR = 4.09) and healthy controls (P < 0.0001; OR = 2.79). Irrespective of the level of clinical severity, blood group 'B' was significantly associated with cerebral malaria (P < 0.0001; OR = 5.95), multi-organ dysfunction (P < 0.0001; OR = 4.81) and non-cerebral severe malaria patients (P = 0.001; OR = 3.02) compared to the uncomplicated category. Prevalence of 'O' group in uncomplicated malaria (P < 0.0001; OR = 2.81) and healthy controls (P = 0.0003; OR = 2.16) was significantly high compared to severe malaria. Meta-analysis of previous studies, including the current one, highlighted the protective nature of blood group 'O' to severe falciparum infection (P = 0.01). On the other hand, carriers of blood group 'A' (P = 0.04) and 'AB' (P = 0.04) were susceptible to malaria severity.

Conclusions: Results of the current study indicate that blood group 'O' is associated with reduced and 'B' blood group with increased risk of development of severe malaria in Odisha, India. Meta-analysis also supports the protective nature of blood group 'O' from severe falciparum infection.

Keywords: ABO blood group, severe malaria, cerebral malaria, multi-organ dysfunction, non-cerebral severe malaria, uncomplicated malaria, meta-analysis

Background

Malaria is an infection caused by protozoan parasites of the genus Plasmodium and transmitted by the bite of infected Anopheles mosquitoes. Out of the four species that infect humans, Plasmodium falciparum is the principal cause of severe clinical manifestations [1]. Cyto-adherence and rosetting are important components of several possible pathogenic mechanisms attributed to the cause of severe infection [2]. An association between ‘O’ blood group and lower rosetting capacity has been demonstrated [3]. However, rosetting capacities of blood group ‘A’, ‘B’ or ‘AB’ have remained controversial [4-7]. On the erythrocyte surface, the A and B antigens are tri-saccharides -A, GalNAc1-3(Fucα1-2)Gal1β1; and B-Gal1α1-3(Fucα1-2)Galβ1 respectively, that are attached...
to different glycolipids and glycoproteins [8]. An enzyme glucotransferase is necessary for the production of A and B antigens. On the other hand, blood group ‘O’ carries a disaccharide H antigen (Fuc(1→2)Galβ1) due to the absence of the enzyme glucotransferase [8]. Variations in gene encoding functional glucotransferase have been associated with protections from severe *P. falciparum* malaria [9] and this observation has been further strengthened by a recent genome wide association study [10]. Tri-saccharide of ‘A’ and ‘B’ blood group is presumed to act as receptors and functions as an important factor for rosetting [7]. However, RBCs of blood group ‘O’ do not express tri-saccharide, and rosettes formed by infected ‘O’ blood group RBCs are smaller and easily disrupted compared to blood groups A, B or AB [4,7,11].

There are limited reports in literature on association of ABO blood group and susceptibility to severe falciparum malaria. The association of blood group ‘AB’ and severe malaria has been demonstrated in various populations, viz. Sri Lanka [12], Mali [11], and Ethiopia [1], while a significant association has also been reported between blood group ‘A’ and severity in Gabon [13], Ethiopia [1] and Zimbabwe [14]. The role of blood group ‘B’ and severe falciparum infection has not been reported.

Malaria remains a major health problem in India. The National Vector Borne Disease Control Programme (NVBDCP), India, has reported that 1.8 million cases of malaria and 1,000 malaria-related deaths occur annually [15]. However, the World Health Organization (WHO) estimates that figure to be 20 million cases and 15,000 deaths [16]. A recent study reported a staggering 1,22,000 deaths due to malaria in India, and Odisha as a major contributor to this mortality [17]. Although the state is hyper-endemic to *P. falciparum* malaria [18] and contributes 29.8% of deaths related to the infection [15], no study has been carried out in the local population to assess the association of ABO blood group in severe infection. Therefore, the current study aims to investigate this association and look into the overall role of ABO blood group in risk/resistance to the development of severe malaria by a meta-analysis of results from the current study and earlier published reports.

**Methods**

**Study site and participants**

The study was conducted at S.C.B. Medical College, Cuttack, Odisha, India between 2008-2009. Patients (age ≥15 years) admitted to the Department of Medicine with a short history of fever were clinically examined in detail and screened for *P. falciparum* infection by Giemsa-stained thick and thin blood smears and immune chromatography test (SD Bio Standard Diagnostics India). Detection of *P. falciparum* was also performed by nested polymerase chain reaction (PCR). Individuals infected only with *P. falciparum* were included. Clinical categorization was done based on WHO guidelines [19]. Uncomplicated malaria (UM) was defined as patients with fever and evidence of falciparum infection in the blood. Severe malaria(SM) was categorized into three groups based on distinct clinical features: 1) Cerebral malaria (CM), 2) Non cerebral severe malaria (NCSM) and 3) Multi-organ-dysfunction (MOD). CM was further defined as patients with altered sensorium, GCS (Glasgow Coma Scale) of ≤ 10. NCSM patients had one of the several manifestations of severe malaria without cerebral involvement, namely severe anaemia (haemoglobin <5 g/dl), acute renal failure (serum creatinine >3 mg/dl), jaundice (serum bilirubin >3 mg/dl), acute respiratory distress syndrome (PaO2/FIO2 <200), haemoglobinuria (dark red or black coloured urine positive for haemoglobin) and shock (systolic BP of <80 mm Hg). MOD was diagnosed based on presence of two or more organ involvement like CNS (GCS≤10), respiratory (PaO2/FIO2 <200), renal failure (serum creatinine >3 mg/dl) and hepatic dysfunction (ALT/AST >3 times of normal, prolonged prothrombin time and albuminaemia). 174 healthy controls (HC) of identical ethnicity and hailing from a similar geographical background were enrolled. None of the controls reported history of clinical malaria in the last 5 years. They were essentially healthy and negative for demonstrable *P. falciparum* infection. The risk of exposure to malaria was similar for both HC and patients. Criteria for analysis of mortality: Since death occurred in the CM and MOD groups’ only patients from these groups were included for analysis. The study was approved by the Institutional Ethics Committee of the Medical College and blood samples were collected after written consent of the patients or accompanying person, depending on the clinical scenario.

**Polymerase chain reaction**

Genomic DNA was extracted by GenElute™ Blood Genomic DNA Kit (SIGMA) from whole blood according to the manufacturer’s instructions. Polymerase chain reaction (PCR), has been used to detect up to species level - falciparum, vivax, ovale and malariae [20,21]. In the population under study, *P. falciparum* is the major cause of malaria (>80%) followed by *P. vivax* (10-15%) [22]. With slight modification, genus and species specific nested PCR technique was used to detect *P. falciparum* and/or *P. vivax* infections [20]. In brief, primary PCR (to detect *Plasmodium* genus) was performed in a 20 μl PCR reaction containing 3 μL genomic DNA, 1× Taq buffer containing MgCl2 (SIGMA), 250 μM dNTP (SIGMA)), 200 nM of two genus-specific primer rPLU1
A total of 353 falciparum-infected patients were enrolled in the present study, including 247 severe (SM) and 106 uncomplicated patients (UM). Severe *P. falciparum* malaria patients were further subdivided into cerebral malaria (CM) (n = 59), multi-organ dysfunction (MOD) (n = 80) and non-cerebral severe malaria (NCSM) (n = 108). 174 healthy controls (HC) were included. The mean age between all clinical categories and HC was comparable. Significantly higher levels of haemoglobin were observed in HC than UM and other clinical categories of severe malaria (P < 0.0001) (Table 1).
Blood group B and prognosis

Since the study revealed a significant association of ABO blood group and severe falciparum malaria, possible role of ABO blood groups in disease outcome was explored. Prevalence of blood groups were analysed among subjects who died during treatment and those who survived. Out of 247 cases of severe malaria, twenty patients died during course of treatment: five patients from CM category (n = 59), 15 patients from MOD (n = 80) and none from NCSM (n = 108). Therefore, NCSM patients were not included in the analysis. Furthermore, fifteen patients from CM and MOD groups left the hospital against medical advice, which necessitated their exclusion since their survival status was not known. As a result, a total of 124 patients in CM and MOD groups were analysed. Although frequency of blood group ‘B’ was higher in patients who died (80%) compared to those who survived (54%), the difference was not statistically significant (P = 0.06). Distribution of other blood groups in patients who died and survived was comparable (Table 4).

Studies included in meta-analysis

Nineteen relevant publications were identified on falciparum malaria and ABO blood group. The three primary criteria for inclusion in this meta-analysis were as follows: 1) study must be case-controlled, 2) patients placed under well defined categories as severe and uncomplicated, 3) the sample size should be more than 50 in each category. Out of nineteen studies only five publications satisfied all inclusion criteria and were considered for meta-analysis. Data from the recent study was also included for analysis. Table 5 shows characteristics of all six studies.

Evaluation of publication bias

A funnel plot has been used to test publication bias in meta-analysis. The funnel plot remains symmetric in

### Table 1 Details of study participants

| Subjects | Severe *P. falciparum* malaria clinical categories (SM) | UM | HC | P value |
|----------|-------------------------------------------------------|----|----|---------|
|          | CM | MOD | NCSM |      |      |      |      |      |
| Total number (n) | 59 | 80 | 108 | 106 | 174 | NA |
| Sex (M/F) | 42/17 | 65/15 | 91/17 | 84/22 | 134/40 | NA |
| Mean age in years (range) | 33.5 (15-65) | 33.5 (15-70) | 34 (15-65) | 32.5 (15-80) | 31.1 (16-75) | NS |
| Mean Hg (g/dl)±SD | 9.9 ± 1.9 | 9.9 ± 2.2 | 9.7 ± 2.5 | 10.82 ± 1.9 | 12.8 ± 1.9 | < 0.0001 |

SM: severe malaria; CM: cerebral malaria; MOD: multi-organ dysfunction; NCSM: non-cerebral severe malaria; UM: uncomplicated malaria; HC: healthy control; NA: not applicable; NS: not significant

### Table 2 Prevalence of ABO blood group in different *P. falciparum* malaria clinical categories and healthy controls

| Blood group | SM (n = 247) | UM (n = 106) | HC (n = 174) | SM vs UM P value, OR (95% CI) | SM vs HC P value, OR (95% CI) | UM vs HC P value, OR (95% CI) |
|-------------|--------------|--------------|--------------|-------------------------------|-------------------------------|-------------------------------|
| O           | 63 (25)      | 52 (49)      | 74 (42)      | ref                           | ref                           | ref                           |
| A           | 48 (19)      | 20 (19)      | 36 (21)      | 0.04, 0.50 (0.26 to 0.95)     | 0.12, 0.63 (0.36 to 1.10)     | 0.51, 1.26 (0.65 to 2.42)     |
| B           | 119 (49)     | 24 (23)      | 50 (29)      | <0.0001, 4.09 (2.30 to 7.25)  | <0.0001, 2.79 (1.74 to 4.47)  | 0.23, 1.46 (0.80 to 2.67)     |
| AB          | 17 (7)       | 10 (9)       | 14 (8)       | 0.52, 0.71 (0.30 to 1.69)     | 0.42, 0.70 (0.32 to 1.53)     | 1.00, 0.98 (0.40 to 2.38)     |
| Non O (A+B+AB) | 184 (75) | 54 (51)      | 100 (58)     | <0.0001, 2.81 (1.74 to 4.52)  | 0.0003, 2.16 (1.42 to 3.27)   | 0.32, 1.30 (0.80 to 2.11)     |

NOTE: Data are no. (%) of participants unless otherwise specified.

SM: severe malaria; UM: uncomplicated malaria; HC: healthy controls; OR: odds ratio; CI: confidence interval

### Table 3 Distribution of ABO blood group in different clinical subtypes of severe falciparum infections and uncomplicated malaria

| Blood group | CM (n=59) | MOD (n=80) | NCSM (n=108) | UM (n=106) | CM vs UM P value, OR (95% CI) | MOD vs UM P value, OR (95% CI) | NCSM vs UM P value, OR (95% CI) |
|-------------|-----------|-----------|--------------|------------|-------------------------------|-------------------------------|-------------------------------|
| O           | 12 (20)   | 18 (22)   | 33 (31)      | 52 (49)    | ref                           | ref                           | ref                           |
| A           | 7 (12)    | 19 (24)   | 22 (20)      | 20 (19)    | 0.57, 0.65 (0.22 to 1.91)     | 0.02, 0.36 (0.15 to 0.83)     | 0.18, 0.57 (0.27 to 1.21)     |
| B           | 33 (56)   | 40 (50)   | 46 (43)      | 24 (23)    | <0.0001, 5.95 (2.62 to 13.52) | <0.0001, 4.81 (2.30 to 10.06) | 0.001, 3.02 (1.56 to 5.83)     |
| AB          | 7 (12)    | 3 (5)     | 7 (6)        | 10 (9)     | 0.10, 0.32 (0.10 to 1.04)     | 1.00, 1.15 (0.28 to 4.66)     | 1.00, 0.90 (0.31 to 2.61)     |
| Non O (A+B+AB) | 47 (80)  | 62 (78)   | 75 (69)      | 54 (51)    | 0.0003, 3.77 (1.80 to 7.90)   | 0.0002, 3.31 (1.73 to 6.34)   | 0.0078, 2.81 (1.25 to 3.82)   |

NOTE: Data are no. (%) of participants unless otherwise specified.

CM: cerebral malaria; MOD: multi-organ dysfunction; NCSM: non-cerebral severe malaria; UM: uncomplicated malaria; OR: odds ratio; CI: confidence interval

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absence of publication bias. For ‘O’, ‘B’ and ‘AB’ blood groups the funnel plots were symmetric. However, plot for blood group ‘A’ was asymmetric. Trim-and-fill technique was used to adjust publication bias of blood group ‘A’, for recomputing the effect size [23].

Association of ABO blood group and severe malaria
Heterogeneity Q test was performed to evaluate inter- and intra-study variations and based upon significance value; different models were used for the meta-analysis. Blood group ‘O’ (P < 0.0001), ‘B’ (P = 0.004) and ‘AB’ (P = 0.037) showed significant heterogeneity and, therefore, a random-effect model was employed. On the other hand, a fixed-effect model was used for association of blood group ‘A’ since the Q-test was not significant (P = 0.120).

Results of meta-analysis are shown in Figure 1. Protection against severe malaria was significantly associated with blood group ‘O’ (P = 0.01). In contrast, blood group ‘A’ and ‘AB’ showed significant association to susceptibility (‘A’: P = 0.04 and ‘AB’: P = 0.04). Although, ‘B’ blood group was significantly associated with severe malaria in the present study, the meta-analysis failed to corroborate that association.

Discussion
Results of the current study suggest that patients with blood group ‘B’ have a four-fold increased risk of developing severe infection. In addition, it also reiterates the observation that ‘O’ blood group was significantly associated with a decreased risk of severe malaria. Other blood groups (‘A’ and ‘AB’) did not show any association.

The role of ABO blood group in malaria has been investigated in various populations, but robust data is limited [24]. This study was an attempt to analyse the association of ABO blood group in large number of adult patients and healthy controls in Odisha, a state, highly endemic for falciparum infection [18]. Higher

Table 4 Association of ABO blood group in treatment outcome patients with severe malaria

| Blood group | Dead (n = 20) | Survivors (n = 104) | P value, OR (95%CI) |
|-------------|--------------|---------------------|---------------------|
| O           | 1 (5)        | 21 (20)             | ref                 |
| A           | 2 (10)       | 18 (17)             | 0.59, 0.42 (0.03 to 5.12) |
| B           | 16 (80)      | 56 (54)             | 0.06, 6.00 (0.74 to 48.13) |
| AB          | 1 (5)        | 9 (9)               | 0.53, 0.42 (0.02 to 7.63) |
| Non O (A+B+AB)| 19 (95)     | 83 (80)             | 0.12, 0.20 (0.02 to 1.64) |

NOTE: Data are no. (%) of participants unless otherwise specified; OR: odds ratio; CI: confidence interval

Table 5 Characteristics of individual studies summarized for the meta-analysis

| Author, Year [Ref] | Population | Sample | Blood Groups |
|-------------------|------------|--------|--------------|
| Al-Yaman et al. 1995 [34] | Papua New Guinea | SM (n = 97) | O 44 (45) A 29 (30) B 10 (10) AB 14 (15) |
|                   |            | UM (n = 156) | 53 (34) 55 (35) 28 (18) 20 (13) |
| Lell et al. 1999 [13]  | Gabon      | SM (n = 100) | O 54 (64) A 27 (27) B Data not available AB Data not available |
|                   |            | UM (n = 100) | 64 (64) 11 (11) Data not available Data not available |
| Pathirana et al. 2005 [12] | Sri Lanka | SM (n = 80) | O 19 (24) A 26 (32.5) B 22 (27.5) AB 13 (16) |
|                   |            | UM (n = 163) | 78 (48) 40 (24) 37 (23) 8 (5) |
| Rowe et al. 2007 [11]  | Mali       | SM (n = 124) | O 26 (21) A 40 (32) B 11 (9) AB 47 (38) |
|                   |            | UM (n = 124) | 55 (44) 31 (25) 9 (7) 29 (24) |
| Tekeste et al. 2010 [1] | Ethiopia  | SM (n = 70) | O 16 (23) A 25 (36) B 15 (21) AB 14 (20) |
|                   |            | UM (n = 140) | 64 (46) 42 (30) 23 (16) 11 (8) |
| Current study     | India      | SM (n = 247) | O 63 (25) A 48 (19) B 119 (49) AB 17 (7) |
|                   |            | UM (n = 106) | 52 (49) 20 (19) 24 (23) 10 (9) |

NOTE: Data are no. (%) of participants unless otherwise specified. SM: severe malaria; UM: uncomplicated malaria;
prevalence of blood group ‘O’ was observed in uncomplicated cases, an indication of its possible protective property against severity as indicated in previous reports [1,11-13]. The mechanism of protection is not clearly understood. It is postulated that the phenomenon of rosetting is one of the mechanisms that contributes to disease severity [2]. This rosetting capacity varies among different blood groups [3]. Lowest rosette formation is observed in blood group ‘O’ individuals. This study also clearly highlights the association of severe malaria with

| Study name          | Statistics for each study | Odds ratio and 95% CI |
|---------------------|---------------------------|-----------------------|
|                     | Odds ratio | Lower limit | Upper limit | Z-Value | p-Value |
| I                   | Al-Yaman et al. 1995     | 1.613     | 0.960       | 2.712    | 1.806     | 0.071   |
|                     | Leil et al. 1999         | 0.660     | 0.375       | 1.164    | -1.435    | 0.151   |
|                     | Pathiranetra et al. 2005 | 0.339     | 0.186       | 0.618    | -3.532    | 0.000   |
|                     | Rowe et al. 2007         | 0.333     | 0.190       | 0.582    | -3.857    | 0.000   |
|                     | Tekeste et al. 2010      | 0.352     | 0.184       | 0.674    | -3.162    | 0.002   |
|                     | Current study            | 0.356     | 0.221       | 0.572    | -4.255    | 0.000   |
|                     | Combined                 | 0.500     | 0.295       | 0.850    | -2.559    | 0.010   |
| II                  | Al-Yaman et al. 1995     | 0.783     | 0.454       | 1.350    | -0.879    | 0.379   |
|                     | Leil et al. 1999         | 2.993     | 1.391       | 6.440    | 2.803     | 0.005   |
|                     | Pathiranetra et al. 2005 | 1.481     | 0.822       | 2.666    | 1.307     | 0.191   |
|                     | Rowe et al. 2007         | 1.429     | 0.821       | 2.486    | 1.262     | 0.207   |
|                     | Tekeste et al. 2010      | 1.296     | 0.706       | 2.381    | 0.837     | 0.403   |
|                     | Current study            | 1.037     | 0.581       | 1.852    | 0.123     | 0.902   |
|                     | Combined                 | 1.278     | 1.002       | 1.630    | 1.978     | 0.048   |
| III                 | Al-Yaman et al. 1995     | 0.525     | 0.243       | 1.137    | -1.634    | 0.102   |
|                     | Pathiranetra et al. 2005 | 1.292     | 0.700       | 2.383    | 0.819     | 0.413   |
|                     | Rowe et al. 2007         | 1.244     | 0.496       | 3.116    | 0.466     | 0.641   |
|                     | Tekeste et al. 2010      | 1.387     | 0.672       | 2.865    | 0.885     | 0.376   |
|                     | Current study            | 3.176     | 1.891       | 5.337    | 4.366     | 0.000   |
|                     | Combined                 | 1.348     | 0.734       | 2.475    | 0.962     | 0.336   |
| IV                  | Al-Yaman et al. 1995     | 1.147     | 0.550       | 2.393    | 0.365     | 0.715   |
|                     | Pathiranetra et al. 2005 | 3.759     | 1.489       | 9.492    | 2.802     | 0.005   |
|                     | Rowe et al. 2007         | 2.000     | 1.152       | 3.472    | 2.461     | 0.014   |
|                     | Tekeste et al. 2010      | 2.932     | 1.253       | 6.857    | 2.481     | 0.013   |
|                     | Current study            | 0.710     | 0.314       | 1.606    | -0.824    | 0.410   |
|                     | Combined                 | 1.747     | 1.014       | 3.008    | 2.011     | 0.044   |

Figure 1: Forest plots of blood groups in association to severe malaria. Meta-analysis was performed including previous reports and current study by comprehensive meta-analysis software. Random or fixed model of meta-analysis was employed for calculation of the combined effect of all studies. Forrest plots evaluating resistance/risk factor of blood group O (I), A (II), B (III) and AB (IV) to severe malaria are shown.
blood group ‘B’. Interestingly, this association was valid across all grades of severity. Although the number of patients who died (n = 20) was small, frequency of blood group ‘B’ was higher in these subjects (80%) in comparison to survivors (54%). Previously, studies on patients from Zimbabwe [14], Gabon [13] and Ethiopia [1] showed a significant association of ‘A’ blood group with severe malaria. Blood group ‘AB’ has also been reported to be associated with severity in Sri Lanka [12], Mali [11] and Ethiopian populations [1]. There are no reports implicating blood group ‘B’ with severity. The variability of observations made with regard to different blood groups may be attributable to different rosetting capacity, heterogenous population groups and varied infective strains [3]. Blood group ‘A’ in Uganda and Gambia [4,7], ‘B’ group in Thailand [4] and ‘AB’ group in Kenya [6] have been associated with increased rosetting phenomenon.

It is presumed that the prevalence of blood group ‘O’ would be higher in malaria endemic areas due to its capacity to confer protection. An analysis of blood group in healthy controls revealed a distribution of ‘O’ (42%), which was much higher compared to ‘A’ (21%), ‘B’ (29%) and ‘AB’ (8%). A community-based study in a tribal population of Odisha, where malaria is endemic, also showed higher prevalence of blood group ‘O’ [25]. Significantly, a lower prevalence ‘O’ blood group has been observed in other malaria non-endemic states like Maharashtra [26,27] and Uttar Pradesh [28] in India, indicating a selective advantage of this blood group in endemic localities. This hypothesis is further supported by higher prevalence of ‘O’ blood group worldwide where malaria infection is prevalent [29].

Meta-analysis combines results of several similar studies to produce a single estimate of the major effect with enhanced precision [30]. Current analysis revealed significant association of blood group ‘O’ with protection against severe malaria (OR = 0.45). In contrast, blood groups ‘A’ and ‘AB’ were associated with susceptibility to severity: Blood group ‘A’ and ‘AB’ conferred 1.27- and 1.74-fold higher risks respectively. Although in the current study, ‘B’ blood group was significantly associated with severe malaria on meta-analysis the association was insignificant. This variation may be a population specific phenomenon.

There are however limitations in the present study. Several RBC polymorphisms, including those linked to glucose-6-phosphate dehydrogenase, pyruvate kinase, complement receptor-1 and haemoglobinopathies, have a role in the clinical outcome of malaria [31], but were not included for analysis in the present study. Such polymorphisms have only been reported from the Western belt of Odisha among tribal communities [32,33] and not in areas from which patients and HC in the current study were enrolled.

Conclusion
This study reveals a significant association of blood group ‘B’ to severe malaria. The association is valid across all grades of severity. Blood group ‘O’ confers protection to severe disease. The meta-analysis reiterates the observation of protection conferred by blood group ‘O’ and highlights susceptibility of group ‘A’ and ‘AB’ to severe malarial infection.

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Authors’ contributions
ARP was involved in detection of malaria by PCR, analysis, interpretation, performed statistics and writing the first draft of the manuscript. SKP was also involved in species-specific PCR and analysis. ANS, RT, BR and BKD made a contribution in the design, data interpretation, work supervision and critically revising the manuscript. All authors read and approved the manuscript.

Competing interests
The authors declare that they have no competing interests.

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References
1. Tekeste Z, Petros B. The ABO blood group and Plasmodium falciparum malaria in Awash, Metehara and Ziway areas, Ethiopia. Malar J 2010, 9:280.
2. Ringwald P, Peyron F, Lepers JP, Rabarison P, Rakotomalala C, Razanampanarany M, Rabodonirina M, Roux J, Le Bras J. Parasite virulence factors during falciparum malaria: rosetting, cytoadherence, and modulation of cytoadherence by cytokines. Infect Immun 1993, 61:198-5204.
3. Uneke CJ. Plasmodium falciparum malaria and ABO blood group: is there any relationship? Parasitol Res 2007, 100:759-765.
4. Carlson J, Wahlgren M. Plasmodium falciparum erythrocyte rosetting is mediated by promiscuous lectin-like interactions. J Exp Med 1992, 176:1311-1317.
5. Udomsangperch T, Todd J, Carlson J, Greenwood BM. The effects of hemoglobin genotype and ABO blood group on the formation of rosettes by Plasmodium falciparum-infected red blood cells. Am J Trop Med Hyg 1993, 48:149-153.
6. Rowe A, Obeiro J, Newbold CI, Marsh K. Plasmodium falciparum rosetting is associated with malaria severity in Kenya. Infect Immun 1995, 63:2329-2336.
7. Barragan A, Kremsner PG, Wahlgren M, Carlson J. Blood group A antigen is a coreceptor in Plasmodium falciparum rosetting. Infect Immun 2000, 68:2971-2975.
8. Daniels G. The molecular genetics of blood group polymorphism. Transpl Immunol 2003, 14:143-153.
9. Fry AE, Griffiths MJ, Auburn S, Diakite M, Forton JT, Green A, Richardson A, Wilson J, Jallow M, Siyoo-Jof, F J, Pinder M, Peshu N, Williams TN, Marsh K, Molynieux ME, Taylor TE, Rockett KA, Kwiatkowski DP. Common variation in the ABO glycosyltransferase is associated with susceptibility to severe Plasmodium falciparum malaria. Hum Mol Genet 2008, 17:567-576.
10. Jallow M, Teo YY, Small KS, Rockett KA, Deloukas P, Clark TG, Kiven K, Bongar NA, Conley DJ, Pinder M, Siyoo-Jof, F J, Usen S, Aserum S, Limbong MG, Caffey A, Dunham A, Fry AE, Green A, Gwilliam R, Hunt SE, Inoue M, Jeffreys AE, Mandy A, Pakiotie A, Potter S, Ragouissi J, Rogers J, Rowlands K, et al. Genome-wide and fine-resolution association analysis of malaria in West Africa. Nat Genet 2009, 41:657-665.
11. Rowe JA, Handel IG, Thera MA, Deans AM, Lyke KE, Kone A, Diallo DA, Raza A, Kajiyama T, Marini K, Plowe CV, Dungbo OK, Moulds JM. Blood group O protects against severe Plasmodium falciparum malaria through the mechanism of reduced rosetting. Proc Natl Acad Sci USA 2007, 104:17471-17476.
12. Pathirana SL, Alles HK, Bandara S, Phone-Kyaw M, Perera MK, Wickremasinghe AR, Mendis KN, Handunnetti SM. ABO-blood-group types and protection against severe, Plasmodium falciparum malaria. Ann Trop Med Parasitol 2005, 99:119-124.
13. Leil B, May J, Schmidt-Ritt RJ, Lehman LG, Lucker D, Greve B, Matoucek P, Schmidt D, Hetzbich K, Mckenrahaup FP, Moyer CG, Bierleu J, Kiemmmer PG. The role of red blood cell polymorphisms in resistance and susceptibility to malaria. Clin Infect Dis 1999, 28:794-799.
14. Fischer PR, Boone P. Severe malaria associated with blood group. Am J Trop Med Hyg 1998, 58:122-123.
15. National Vector Borne Disease Control Programme: Ministry of Health and Family Welfare. Government of India Report 2004.
16. WHO: World Malaria Report 2009 Geneva.
17. Dhingra N, Jha P, Sharma VP, Cohen AA, Jotkar RM, Rodriguez PS, Bassani DG, Suraweera W, Laxminarayan R, Petro R. Adult and child malaria mortality in India: a nationally representative mortality survey. Lancet 2010, 376:1768-1774.
18. Sharma SK, Chattopadhyay R, Chakrabarti K, Pati SS, Srinavasta VK, Tyagi PK, Mahanty S, Misra SK, Adak T, Das BS, Chitnis CE. Epidemiology of Malaria transmission and development of natural immunity in a malaria-endemic village, San Dulakudar, in Orissa state, India. Am J Trop Med Hyg 2004, 71:457-465.
19. World Health Organization, Communicable Diseases Cluster. Severe falciparum malaria. Trans R Soc Trop Med Hyg 2000, 94(Suppl 1):1-90.
20. Singh B, Bobogare A, Cox-Singh J, Shounouguo G, Abdullah M, Raebek H: A genus- and species-specific nested polymerase chain reaction reaction malaria detection assay for epidemiologic studies. Am J Trop Med Hyg 1999, 60:687-692.
21. Shounouguo G, Vinyaskol S, Juma W, Thaitong S, Brown KN. Identification of the four falciparum parasites in field samples by using the polymerase chain reaction and detection of a high prevalence of mixed infections. Mol Biochem Parasitol 1993, 58:283-292.
22. Ranjit MR. The epidemiology of malaria in Orissa. Indian Council of Medical Research Bulletin 2006, 36:29-38.
23. Duval S, Tvedre R: Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biom metrics 2000, 56:455-463.
24. Lascertales MP, Owens S, O'Donnell J, Bunj J, Bosch-Capblanch X, Brabin BJ. ABO blood group phenotypes and Plasmodium falciparum malaria: unlocking a pivotal mechanism. Adv Parasitol 2007, 63:1-30.
25. Balgir RS. Genetic heterogeneity of population structure in 15 major scheduled tribes in central-eastern India: A study of immune-hematological disorders, Indian J Hum Gen 2006, 12:86-92.
26. Chavan A, Pawar S, Bag M. Allelic frequency of ABO and Rh D blood group among the Banjara backward caste of Yavatmal District, Maharashtra, India. Nature Precedings 2010.
27. Warghat NE, Sharma NR, BAG MM, Tada R, Sharma SH, Fachlore GS: ABO and Rh blood group distribution among Kunbis (Maratha) population of Amravati District, Maharashtra. Nature Precedings 2010.
28. Rai V. Genetic analysis of ABO and Rh blood groups in Brahmin population of Uttar Pradesh, India. Nature Precedings 2011.
29. Cserti CM, Dzik W: The ABO blood group system and Plasmodium falciparum malaria. Blood 2007, 110:2250-2258.