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Severe Imported Malaria in a Serbian Referral Center

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Abstract

Background: World Health Organization estimates that 3.2 billion people are at risk of being infected with malaria. Thus, adequate diagnostic protocols for malaria, especially those aimed at determining disease severity, are paramount in both endemic and non-endemic settings.

Methods: We analyzed 22 patients with severe malaria and compared their clinical and laboratory findings with those of patients with non-severe malaria in search of predictors of disease severity. All patients were treated at the Infectious and Tropical Diseases University Hospital, Clinical Centre of Serbia in Belgrade, Serbia from 2000 to 2010.

Results: Average age was 44.86 ± 12.33 years and men predominated (95.45%) among patients with severe malaria. Patients with severe malaria were infected with *P. falciparum* significantly more frequently compared with those with non-severe disease (p=0.047). Jaundice was the most commonly observed feature of severe malaria, followed by anemia and renal failure. A multifactor analysis of variance showed that thrombocytopenia (p=0.05) and high serum TNF-alpha levels (p=0.02) were significantly associated with disease severity.

Conclusion: A high index of suspicion for malaria should be maintained when evaluating febrile patients returning from malaria endemic regions. Elevated serum TNF-alpha levels and thrombocytopenia are associated with severe malaria in non-endemic settings.

Keywords: Malaria; Serbia; Thrombocytopenia; Tumor necrosis factor-alpha

Introduction

World Health Organization (WHO) estimates that some 3.2 billion people (about 44% of the world's population) are at risk of being infected with malaria and developing the disease [1]. Some 214 million cases of malaria were reported in 2015 and resulted in 438 000 deaths [2]. The WHO African region carries a disproportionately high share of the global malaria burden, considering it was home to 88% of malaria cases and 90% of malaria deaths in 2015 [2].

Severe malaria is defined by clinical or laboratory evidence of vital organ dysfunction [3]. In 1990, WHO established the criteria for severe malaria in order to facilitate future clinical and epidemiological studies [4]. In the year 2000, these criteria were revised to include other clinical and laboratory abnormalities that portend a poor prognosis based on clinical experience in semi-immune patients [5]. *Plasmodium falciparum* is the most common cause of severe malaria, but *Plasmodium vivax* and *Plasmodium knowlesi* can also cause severe disease [6]. Although rare, *Plasmodium ovale* has also been reported in patients with severe malaria [7].

The circulating level of TNF-alpha was shown to be a marker of organ failure and, as such, was correlated to malaria severity [8-10]. Low thrombocyte counts were also proven to be related to the severity of both vivax and falciparum malaria, although some authors questioned their usefulness for triage and prognostication [11-13]. Most studies correlating platelet counts and TNF-alpha levels to disease severity were conducted in endemic settings [9-13].

In 1975, WHO announced that malaria was eradicated from Europe, with what was then the Socialist Federal Republic of Yugoslavia designated as malaria free since 1964. This meant that malaria was also eradicated from Serbia which was one of the six republics constituting the federation [14]. However, imported malaria remained a concern in the years to follow [15].

The aim of this study was to identify the demographic, parasitological, clinical and laboratory characteristics associated with severe malaria in a non-endemic setting.

Materials and Methods

We conducted a case control study in order to analyze the clinical, laboratory and parasitological characteristics of severe malaria in the Republic of Serbia.

Researchers browsed through archived paper-based medical records and identified all patients that were treated for malaria at Infectious and Tropical Diseases University Hospital, Clinical Centre of Serbia in Belgrade, Serbia in an 11 year period (2000-2010).

Infectious and Tropical Diseases University Hospital is a tertiary health care facility that treats patients with infectious and/or tropical diseases that cannot be diagnosed and/or treated in other hospitals in Serbia and patients who reside in the capital city of Belgrade and present directly to the clinic.

Researchers then analyzed the selected medical records and determined which subset of malaria patients met the criteria for severe disease.

Severe malaria was defined according to WHO criteria as the presence of one of the following in a patient with a parasitological diagnosis of malaria: hyperparasitemia (more than 5% parasitized erythrocytes), shock, abnormal bleeding, pulmonary edema/ARDS, jaundice (a bilirubin concentration higher than 50 μmol/L), renal failure (a urine output <400 ml per 24 hours and a serum creatinine concentration higher than 265 μmol/L), severe anemia (a hemoglobin concentration less than 7 g/dL or a hematocrit less than 20%), hemoglobinuria, impaired consciousness (a Glasgow coma score less than 11), prostration, multiple convulsions (at least two convulsions in 24 hours), acidosis (a bicarbonate concentration less than 15 mmol/l or arterial/capillary pH lower than 7.25), hyperlactatemia (an arterial lactate concentration >5 mmol/L) hypoglycemia (a plasma glucose concentration lower than 2.2 mmol/L) [5]. Patients with non-severe malaria were used as controls.

Findings on physical examination, parasitological and immunological investigation and blood chemistry panel and complete blood count results were entered into a Microsoft Excel 2010 document.

We determined the TNF-alpha concentration in the patients' serum using an ELISA-based standardized kit called the Quantikine ELISA Kit (R&D Systems) and examined thick and thin peripheral blood stained with Giemsa. Parasitemia was expressed as a percentage of parasitized erythrocytes.

Statistical analysis was preformed using IMB's SPSS Statistics v14 utilizing the methods of descriptive statistics, the chi-squared test, Fisher's exact test (where assumptions for chi-squared test were not met) and multivariate analysis of variance (MANOVA).

We analyzed average patient age, malaria chemoprophylaxis compliance, immunity to malaria, the presence of comorbidities, symptoms duration before admission to hospital, severe thrombocytopenia (platelet count <50,000) and the TNF-α level as possible predictors of severe malaria using MANOVA.

The authors obtained ethical approval from the Ethical committee of School of Medicine, University of Belgrade.

Results

We identified 103 patients treated for malaria at Infectious and Tropical Diseases University Hospital, Clinical Centre of Serbia from 2000 to 2010.

Age (years)	Number of patients		Percentage of patients (%)	
	Non-severe	Severe	Non-severe	Severe
0 - 10	1	0	1.23	0
11 - 20	1	0	1.23	0
21 - 30	6	3	7.41	13.64
31 - 40	14	4	17.28	18.18
41 - 50	22	6	27.16	27.27
51 - 60	24	8	29.63	36.36
61 - 70	13	1	16.05	4.55
Total	81	22	100	100

Table 1: Age distribution.

A subgroup of 22 patients (21.35%) met the criteria for severe malaria at presentation. Men predominated (95.45%) and average patient age was 44.86 ± 12.33 years (range 21-61 years).

The age distribution of patients with severe malaria is represented in Table 1. It was not statistically significantly different than the age distribution of patients with non-severe malaria (Fisher's exact test, p=0.759).

A single patient fulfilled five criteria for severe malaria and suffered a lethal outcome (Table 3). No patient met more than five criteria. We did not analyze the correlation between the number of fulfilled criteria and disease outcome due to the sparsity of data.

Plasmodium species	Number of patients		Percentage of patients (%)	
	Severe	Non-severe	Severe	Non-severe
Plasmodium falciparum	19	56	86.38	69.13
Plasmodium falciparum + vivax	1	4	4.54	4.94
Plasmodium vivax	1	19	4.54	23.46
Plasmodium ovale	1	0	4.54	0
Plasmodium malariae	0	2	0	2.47
Total	22	81	100	100

Table 2: Patient distribution by *Plasmodium* species.

The three patients that suffered a fatal outcome all had severe malaria making the overall lethality of malaria 2.91% and the lethality of severe malaria 13.64%. The majority of patients with severe malaria were infected with *P. falciparum* (90.9%) which is a statistically

significant difference compared with patients with non-severe forms of the disease (Fisher's exact test, p=0.047).

Number of fulfilled criteria	Number of patients	Percentage of patients (%)
1	13	59.10
2	4	18.19
3	3	13.63
4	1	4.54
5	1	4.54
Total	22	100

Table 3: Distribution of patients with severe malaria according to the number of fulfilled WHO criteria.

We identified *P. vivax* in the blood of 2 patients with severe malaria, whereas one patient had malaria caused by *P. ovale*. No patients with severe malaria were infected with *P. malariae*.

A vast majority of patients with severe malaria (95.45%) had a single *Plasmodium* species as the causative agent. One patient had a mixed *P. falciparum* and *P. vivax* infection (Table 2).

Thirteen patients (59.10%) fulfilled only one criteria for severe malaria at presentation, while 9 (40.90%) had two or more criteria.

Patient distribution according to the features of severe malaria is represented in Table 4.

Jaundice was present in 28.21% of patients making it the most commonly fulfilled criterion for severe malaria in our cohort (χ^2=22.744, p=0.05).

Features of severe malaria (WHO criteria)	Number of patients	Percentage of patients (%)
Cerebral malaria	3	7.69
Pulmonary edema/ARDS	2	5.12
Renal failure	4	10.26
Disseminated intravascular coagulation	1	2.56
Jaundice	11	28.21
Anemia	7	17.95
Haemoglobinuria	1	2.56
Hyperparasitemia	10	25.65

Table 4: Distribution of patients according to the features of severe malaria.

No criteria of disease severity aside from those represented in Table 4 were fulfilled by patients in the study cohort. Two patients with severe malaria had arterial hypertension, while one had diabetes mellitus. The rest had no comorbidities.

The average TNF-alpha serum level was 31.91 pg/ml. Fourteen patients (63.63%) had severe thrombocytopenia (less than 50×10^9 thrombocytes/L). The MANOVA revealed that patients with severe malaria had statistically significantly higher levels of TNF-α (p=0.02) and statistically significantly higher frequency of severe thrombocytopenia (p=0.05) compared with patients with non-severe malaria (Table 5).

Variables	Non-severe malaria	Severe malaria	F
Age (years)	47.47	43.68	0.48
Lack of Chemoprophylaxis (% of patients)	75.31	77.28	1.69
Absence of Immunity (% of patients)	46.92	50.00	0.84
Comorbidities (% of patients)	23.45	13.63	0.15
Duration of symptoms (days)	6.47	7.23	0.03
Serum TNF-α level (pg/mL)	17.12	31.91	5.79
Number of platelets <50.000 (% of patients)	18.52	63.63	4.10

Table 5: Multivariate analysis of variance (MANOVA).

Discussion

Data is lacking when it comes to the severe forms of malaria in countries where the disease is not endemic because most studies can include only a small number of patients [16]. The results of our study show that 21.35% of patients had severe malaria according to WHO criteria. Other authors have found that the proportion of patients with severe disease varies from 1 to 38% of the total number of patients with malaria [17]. Variations in the percentage of patients with the severe form of malaria are best illustrated by the following: the disease was severe in 7.5% of patients with malaria in Canada, in 15.9% in the US and 16% in the UK [18-20]. In Germany, 27.9% of patients with malaria caused by *P. falciparum* had severe disease [21].

The vast majority of patients in our study (approximately 91%) had malaria caused by *P. falciparum*, which is in concordance with data from other studies. Both this and other studies have indicated that severe malaria can be caused by *P. vivax*, the so called "falciparum like" syndrome [22-25]. No cases of severe *P. knowlesi* malaria were reported in Serbia in the analyzed time span. In our study, *P. falciparum* was significantly over-represented in the subgroup of patients with severe malaria compared with those with non-severe malaria.

This study shows that the most common features of severe malaria are the following (listed by decreasing frequency): jaundice, hyprparasitemia, anemia, renal failure, cerebral malaria, pulmonary edema/ARDS. Haemoglobinuria (also called blackwater fever) and disseminated intravascular coagulation (DIC) occurred in one patient each. According to research by authors from Germany and Spain, hyperbilirubinemia and hyperparasitemia were most commonly associated severe malaria [21,26]. A multicentric study from Thailand showed that jaundice was present in 529 of 1050 patients (50.4%) with severe malaria and a hyperparasitemia was present in 33.3% [27].

Cerebral malaria, renal failure, ARDS, anemia and DIC were most commonly associated with a fatal outcome in the US [28]. In this study, fatal outcomes occurred in 3 patients (2.91%) the immediate causes of death being cerebral malaria, renal failure and pulmonary edema/ARDS.

Many studies have shown that increasing age is a risk factor for severe malaria although some authors have questioned this view [29-33]. The average age of patients with severe malaria in this study was approximately 44 years and statistical analysis lead us to the conclusion that old age was not a risk factor for severe malaria. The lack of chemoprophylaxis is the second most commonly cited risk factor for severe malaria [29,34,35]. Extensive research that had been conducted in France form 1996 to 2003 and included the results from 120 reference laboratories analyzed at the National Center for imported and autochthonous malaria, showed an association between the severe malaria and increasing age, lack of chemoprophylaxis and duration of symptoms before diagnosis [34]. No such associations were proven in this study. The absence of acquired immunity to malaria was not a risk factor for the development of severe disease; a finding similar to those of French authors [36]. Moreover, previous research suggests that congenital immunity is of greater importance, and that acquired immunity depends on the long-term exposure to malaria parasites making it generally limited to areas of high endemicity [37].

According to the results of our study, patients with severe malaria had significantly higher TNF-α levels and a significantly higher frequency of severe thrombocytopenia compared with patients with non-severe disease. A retrospective study that had been conducted at The University Hospital in Heidelberg, Germany and included 122 patients with falciparum malaria, showed that thrombocytopenia was a significant predictor of severe malaria [21]. These findings were corroborated by other authors [20,38]. In severe malaria, the concentrations of proinflammatory cytokines such as TNF-α, IL-1, IL-6, and IL-12 are elevated [39]. The high level of TNF-α in patients with falciparum malaria correlates with disease severity, hypoglycemia, hyperparasitemia, jaundice, renal failure, cardiovascular complications and death [40]. Many studies have shown a statistically significant correlation between severe malaria and TNF-α levels, the presence of hyperparasitemia, jaundice and acute renal failure [41,42].

Conclusion

Even though it is a rare cause of morbidity in a non-endemic setting, a high index of suspicion for malaria should be maintained when evaluating febrile patients returning from malaria endemic regions. TNF-alpha is significantly higher and thrombocytes are significantly lower in patients with severe malaria in both endemic and non-endemic settings and Serbia is no exception. Other proinflammatory cytokines may also represent a viable early diagnostic test for predicting malaria severity and present us with an avenue of future research. Since *P. falciparum* causes a large proportion of imported malaria cases in Serbia and is most strongly associated with severe disease (lethal in one in eight patients), the identification of *Plasmodium spp.* in a patient's blood or even a febrile illness in a patient returning from a *P. falciparum* endemic region, should prompt the clinician to request a determination of TNF-alpha levels and platelet counts in order to take measures to prevent and/or more effectively treat possible organ failure. Whether this approach is cost-effective remains to be elucidated.

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