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VARIATION OF NITRITE, NITRATE, AND METHAEMOGLOBIN LEVELS IN SEVERE MALARIA PATIENTS: A PROSPECTIVE STUDY

Biochemistry

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ABSTRACT

Background: Severe malaria, caused by Plasmodium parasites, poses a significant public health challenge with high mortality rates, especially in endemic regions. Nitric oxide (NO) and its derivatives, nitrite, nitrate, and methemoglobin, play crucial roles in the pathophysiology of severe malaria, affecting vascular function and oxidative stress. **Aims And Objectives:** This prospective observational study aimed to investigate changes in nitrite, nitrate, and methemoglobin levels in severe malaria patients over ten days, providing insights into disease progression and potential biomarkers for disease severity. **Results:** The study revealed significant increases in serum nitric oxide, nitrate, arginine, methaemoglobin, and creatinine levels from Day 1 to Day 10 of malaria infection. Nitric oxide levels increased dynamically, suggesting its role in modulating host defense. Nitrate levels elevated significantly, possibly as a compensatory response to endothelial dysfunction. Arginine levels rose substantially, indicating increased substrate availability for NO synthesis. Methaemoglobin levels and creatinine levels also showed significant increases, reflecting enhanced oxidative stress and renal dysfunction, respectively. **Conclusion:** The findings underscore the complex interplay between NO metabolism, endothelial function, and oxidative stress in severe malaria. Elevated NO levels may reflect an adaptive immune response, while increased nitrate and arginine levels suggest compensatory mechanisms to maintain vascular homeostasis. The rise in methaemoglobin levels highlights oxidative stress's severity, while elevated creatinine levels signify renal impairment. These results contribute to understanding severe malaria pathophysiology and identify potential therapeutic targets for improving clinical outcomes.

KEYWORDS

Malaria, Nitrite, Nitrate, Methaemoglobin

INTRODUCTION:

Severe malaria, a life-threatening complication of infection by the Plasmodium parasite, is characterized by its ability to cause multi-organ dysfunction and high mortality rates, particularly in regions where Plasmodium falciparum is endemic. Despite significant progress in the development of preventive measures and treatment strategies, severe malaria remains a significant public health challenge, disproportionately affecting vulnerable populations such as children and pregnant women.

Nitric oxide (NO) is a crucial signaling molecule involved in a myriad of physiological processes, including vasodilation, immune modulation, and host defense against pathogens. It plays a pivotal role in regulating vascular tone, maintaining blood pressure, and mediating immune responses to microbial invaders. Nitrite and nitrate, stable end products of NO metabolism, serve as reliable indicators of NO bioavailability and endothelial function. Methemoglobin, a modified form of hemoglobin incapable of oxygen transport, reflects oxidative stress and redox imbalance within the body.

Several studies have underscored the importance of NO in the pathophysiology of severe malaria. Yeo et al. [1] demonstrated impaired NO bioavailability and reversible endothelial dysfunction in adults with falciparum malaria, highlighting the role of NO dysregulation in the pathogenesis of the disease. Additionally, Lopansri et al.[2]observed prominent effects of plasma volume expansion on parasite clearance and amino acid metabolism in severe malaria, suggesting a potential link between NO metabolism and disease severity.

In the context of severe malaria, alterations in nitrite, nitrate, and methemoglobin levels may provide valuable insights into disease pathophysiology and serve as potential biomarkers for disease severity and prognosis. Elevated levels of nitrite and nitrate may indicate increased NO production and endothelial activation, which have been implicated in the pathogenesis of microvascular dysfunction and organ damage in severe malaria. Pamplona et al. [3] demonstrated that heme oxygenase-1 and carbon monoxide suppress the pathogenesis of experimental cerebral malaria, highlighting the role of oxidative stress and redox balance in modulating disease outcomes.

Understanding the complex interplay between NO metabolism, endothelial function, and oxidative stress in severe malaria is critical for developing novel therapeutic interventions and improving clinical outcomes. Further research is warranted to elucidate the mechanisms underlying NO-mediated pathophysiology in severe malaria and identify potential targets for intervention.

MATERIALS AND METHODS:

Study Design:

This prospective observational study was conducted at the Department of Biochemistry, Seth GSMC & KEMH, Parel, Mumbai, spanning the years 1999 to 2003. The study aimed to investigate changes in nitrite, nitrate, and methemoglobin levels in patients diagnosed with severe malaria over a period of ten days. The study was conducted at the malaria ward of a tertiary care hospital.

Participants:

The study included 30 patients diagnosed with severe malaria who were admitted to the hospital ward. All participants provided informed consent to participate in the study. Patients were assessed on day 1 of admission, and blood samples were collected for biochemical analysis. The same patients were followed up on day 10, and additional blood samples were collected for comparison.

Data Collection:

On day 1 of admission, demographic information, clinical history, and baseline laboratory parameters were recorded for each participant. Blood samples were collected from all patients and processed for the measurement of nitrite, nitrate, and methemoglobin levels using standard laboratory techniques.

Follow-up:

Patients were monitored closely throughout their hospital stay, and treatment was administered according to standard protocols for severe malaria. On day 10, patients were reassessed, and blood samples were collected once again for biochemical analysis.

Sample Collection And Preparation

Blood samples were collected from all participants following overnight fasting. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -80°C until analysis.

Biochemical Analysis

Creatinine: Measured using the Jaffe reaction method with a commercial kit (Roche Diagnostics).
Arginine: Assessed by sakaguchi reaction.
Nitrite and Nitrate: Assessed using the cadmium reduction method of Najwa K Cortas and Nabil W Wakid.
Methemoglobin: Determined by the Evelyn-Malloy method, where methemoglobin levels were quantified by its absorption characteristics in blood.

Statistical Analysis

Data were expressed as mean ± standard deviation. Comparisons

between groups were made using the Student's t-test for normally distributed data, and the Mann-Whitney U test for non-parametric data. A p-value of <0.05 was considered statistically significant.

RESULTS

Table 1. Demographic Criteria

Demographic Criteria	Mean ± SD / Count
Age (years)	32.5 ± 12.7
Gender	
- Male	18
- Female	12
Geographic Origin	
- Urban	15
- Rural	15
Clinical History	
- Previous malaria	10
- Comorbidities	
- Malnutrition	3
- Others	7
Duration of Illness (days)	7.3 ± 2.1
Clinical Presentation	
- Fever	30
- Chills	25
- Headache	20
- Nausea/Vomiting	15
- Jaundice	8
- Altered Consciousness	5
Comorbidities	
- Hypertension	8
- Diabetes	5
Hospitalization History	
- Previous malaria	15
- Other conditions	10
Treatment History	
- Antimalarials used	
- Chloroquine	10
- Artemisinin-based	20

The table presents a comprehensive overview of the demographic and clinical characteristics of the study population consisting of patients diagnosed with severe malaria.

Age And Gender:
The mean age of the patients was 32.5 years with a standard deviation of ±12.7 years. Among the patients, there were 18 males and 12 females, indicating a slightly higher representation of males in the study cohort.

Geographic Origin:
The study included an equal number of patients from urban (15) and rural (15) areas, reflecting a balanced representation of individuals from different geographic settings.

Clinical History:
Ten patients had a history of previous malaria episodes, highlighting the recurrence of the disease in some individuals. Additionally, a subset of patients presented with comorbidities, including malnutrition (3) and other medical conditions (7), underscoring the complexity of managing severe malaria in patients with pre-existing health issues.

Duration of Illness:
The mean duration of illness was 7.3 days with a standard deviation of ±2.1 days, indicating the variability in the duration of illness among the patients.

Clinical Presentation:
Fever was the most common clinical presentation observed in all patients (30), followed by chills (25), headache (20), nausea/vomiting (15), jaundice (8), and altered consciousness (5), illustrating the diverse range of symptoms associated with severe malaria.

Comorbidities:
A subset of patients presented with comorbidities such as hypertension (8) and diabetes (5), suggesting the presence of additional health challenges in some individuals.

Hospitalization History:
Fifteen patients had a history of previous hospitalizations due to malaria, while ten patients had been hospitalized for other medical conditions, indicating a history of recurrent health issues among the study population.

Treatment History:
Regarding antimalarial treatment, chloroquine was administered to 10 patients, while 20 patients received artemisinin-based therapy, highlighting the variation in treatment regimens administered to patients with severe malaria.

Table 2. Variation In Mean And Standard Deviation Of Different Parameters Malaria Day 1 And Malaria Day 10 Group.

Parameter	Day 1 Malaria Group (Group 1) Mean ± SD	Day 10 Malaria Group (Group 2) Mean ± SD	p-value
Nitric oxide	77.30 ± 41.27	111.25 ± 55.64	0.042
Nitrite	35.00 ± 30.88	33.40 ± 19.54	0.732
Nitrate	42.30 ± 25.63	77.15 ± 51.78	0.016
Arginine	24.95 ± 3.49	35.5 ± 2.61	0.001
Methaemoglobin (5 Hb)	10.73 ± 5.13	16.82 ± 5.21	0.008
Creatinine	2.52 ± 0.79	3.09 ± 0.44	0.021

The results of the study comparing the biochemical parameters between the Day 1 and Day 10 malaria groups reveal significant changes over the course of the illness.

Nitric Oxide (NO) Levels: The mean serum nitric oxide levels significantly increased from Day 1 (77.30 ± 41.27 micromole/L) to Day 10 (111.25 ± 55.64 micromole/L) of malaria infection (p = 0.042). Nitrite and Nitrate Levels: There were no significant changes in serum nitrite levels between Day 1 (35.00 ± 30.88 micromole/L) and Day 10 (33.40 ± 19.54 micromole/L) of malaria infection (p = 0.732). However, serum nitrate levels showed a substantial increase from Day 1 (42.30 ± 25.63 micromole/L) to Day 10 (77.15 ± 51.78 micromole/L) (p = 0.016).

Arginine Levels: The mean serum arginine levels significantly increased from Day 1 (24.95 ± 3.49 micromole/L) to Day 10 (35.5 ± 2.61 micromole/L) of malaria infection (p = 0.001).

Methaemoglobin Levels: Methaemoglobin levels significantly increased from Day 1 (10.73 ± 5.13%) to Day 10 (16.82 ± 5.21%) of malaria infection (p = 0.008).

Creatinine Levels: Serum creatinine levels also showed a significant increase from Day 1 (2.52 ± 0.79 mg %) to Day 10 (3.09 ± 0.44 mg %) of malaria infection (p = 0.021).]

DISCUSSION

The comparison of biochemical parameters between the Day 1 and Day 10 malaria groups provides valuable insights into the pathophysiology and progression of severe malaria infection.

Nitric Oxide (NO) Levels: The significant increase in serum nitric oxide (NO) levels from Day 1 to Day 10 (p = 0.042) suggests a dynamic response of the NO pathway during severe malaria infection. NO is known to play a crucial role in modulating vascular tone and host defense against pathogens. The upregulation of NO production may represent an adaptive immune response aimed at controlling parasitemia and preventing microvascular sequestration of infected erythrocytes. This finding is consistent with previous studies demonstrating elevated NO levels in severe malaria and highlights its potential as a biomarker for disease severity.[4]

Nitrite And Nitrate Levels:
While serum nitrite levels remained relatively stable between Day 1 and Day 10 (p = 0.732), there was a significant increase in serum nitrate levels on Day 10 (p = 0.016). Nitrite and nitrate are stable end products of NO metabolism and are indicative of NO bioavailability. The discrepancy in nitrite and nitrate levels may reflect differential regulation of NO synthesis and degradation pathways during the course of malaria infection. The elevated nitrate levels observed on Day 10 may be attributed to increased NO production and subsequent conversion to nitrate, possibly as a compensatory mechanism to maintain vascular homeostasis in the face of endothelial dysfunction.[5]

Arginine Levels:
The substantial increase in serum arginine levels from Day 1 to Day 10 ($p = 0.001$) is noteworthy as arginine serves as a precursor for NO synthesis. The upregulation of arginine levels may reflect increased substrate availability for NO production in response to the heightened immune response during severe malaria infection. Alternatively, arginine supplementation could be a compensatory mechanism to overcome arginine depletion caused by increased NO synthesis.[6]

Methaemoglobin Levels:
The significant increase in methaemoglobin levels from Day 1 to Day 10 ($p = 0.008$) indicates enhanced oxidative stress and redox imbalance during the progression of severe malaria infection. Methaemoglobin, a form of hemoglobin incapable of oxygen transport, is indicative of heme toxicity and oxidative damage to erythrocytes. The elevation in methaemoglobin levels underscores the severity of oxidative stress in severe malaria and its contribution to disease pathogenesis.[7]

Creatinine Levels:
The significant increase in serum creatinine levels from Day 1 to Day 10 ($p = 0.021$) reflects impaired renal function and acute kidney injury associated with severe malaria infection. Acute kidney injury is a common complication of severe malaria, resulting from hemolysis, hypovolemia, and sequestration of infected erythrocytes in the renal microvasculature. The elevation in creatinine levels underscores the importance of monitoring renal function in severe malaria patients to guide appropriate management strategies and prevent further renal damage.

CONCLUSION:

this prospective observational study provides valuable insights into the pathophysiology and progression of severe malaria, focusing on the dynamic changes in nitric oxide (NO) metabolism, endothelial function, and oxidative stress markers. The significant findings include the notable increase in serum nitric oxide levels over the ten-day course of malaria infection, indicating a dynamic response of the NO pathway in severe malaria. Additionally, elevated nitrate levels, along with stable nitrite levels, suggest differential regulation of NO synthesis and degradation pathways during the progression of the disease.

Furthermore, the study highlights the importance of arginine as a precursor for NO synthesis, with a substantial increase observed in serum arginine levels, potentially reflecting an adaptive immune response or compensatory mechanism to support NO production. Moreover, the significant elevation in methaemoglobin levels underscores the role of oxidative stress and redox imbalance in severe malaria pathogenesis, contributing to tissue damage and organ dysfunction.

Importantly, the observed increase in serum creatinine levels reflects impaired renal function and acute kidney injury, emphasizing the need for close monitoring of renal parameters in severe malaria patients to guide appropriate management strategies and prevent further renal damage.

Overall, these findings contribute to a better understanding of the complex interplay between NO metabolism, endothelial function, and oxidative stress in severe malaria, highlighting potential targets for therapeutic intervention and the importance of early detection and management of complications to improve clinical outcomes. Further research is warranted to elucidate the underlying mechanisms and validate the utility of NO-related biomarkers in predicting disease severity and guiding treatment decisions in severe malaria.

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