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Original Research Article

Serum Cortisol Level as a Predictive Biomarker of Severity of Disease among Adult Plasmodium Vivax Patients in Northern India

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Abstract:

Background: Malaria is a major cause of morbidity in Indian subcontinent accounting for approximately 88% of malaria cases in the Southeast Asia. The disease affects all organs and endocrine glands are no exception. Serum cortisol, a hormone released by adrenal gland has the potential to serve as a predictive biomarker for mortality and critical illness among malaria patients.

Aim: This study aimed to investigate changes in serum cortisol levels as a potential biomarker for predicting risk in P. vivax malaria.

Methods: The study was a prospective observational investigation from September 2017 to October 2018. The study involved 40 patients each with complicated, uncomplicated Plasmodium vivax malaria patients and compared with healthy controls. Serum cortisol levels were measured using immunoassay with direct chemiluminescent technology and statistically correlated with Plasmodium vivax malaria infection.

Results: The results revealed that on day 1, there was a significant difference in mean serum cortisol levels between Plasmodium vivax malaria patients and the control group. Cortisol levels were significantly higher in patients with complicated Plasmodium vivax malaria compared to uncomplicated cases on both day 1 and day 7. Cortisol levels remained within the normal range on days 1 and 7 in uncomplicated malaria cases. The optimum cut-off for cortisol level to predict severity of malaria was 18.6 ug/dl for a sensitivity of 88.5% and specificity of 85.8%.

Conclusions: The serum cortisol levels were significantly higher among complicated malaria patients and hence could be a useful indicator for predicting the severity of Plasmodium vivax malaria.

Introduction

Malaria is a major cause of morbidity in Indian subcontinent with 95.5% of population at risk.[1] World Health Organization (WHO) reports that India accounted for approximately 88% of malaria cases in the Southeast Asia and had 8,090 malaria-related deaths in 2019. Malaria is endemic in various parts of India; with the highest transmission occurring in rural and tribal areas.[2] States with a high malaria burden include Odisha, Chhattisgarh, Jharkhand, and the northeastern states. In India, majority of the malaria cases is contributed by Plasmodium vivax and Plasmodium falciparum.[1,2]

Plasmodium falciparum malaria is the main cause of severe and fatal cases of malaria. On the other hand, Plasmodium vivax malaria is typically a milder disease that usually follows a less severe course and rarely leads to death.[3] However, recent reports have challenged this clinical understanding by documenting symptoms, severe manifestations, and even deaths resulting from P. vivax infections alone.

Consequently, P. vivax is no longer regarded as a harmless form of malaria, and it is essential to adopt aggressive treatment measures to prevent complications. Complicated Malaria includes presence of cerebral malaria, acidosis, anaemia, renal failure, respiratory distress, hypoglycaemia, shock, bleeding, hyperparasitemia or jaundice.[4] Malaria affects almost all organs of body. The endocrine glands are no exception with complications such as hypovolemia, electrolyte

imbalance and hypoglycaemia. Cortisol, a hormone released by the adrenal glands during times of stress, plays a crucial role in maintaining vascular tone, endothelial integrity, vascular permeability, and overall homeostasis.[5-9] It also enhances the vasoconstrictor effects of both natural and external catecholamines. Consequently, cortisol levels have the potential to serve as a predictive biomarker for mortality and critical illness among hospitalized patients, independent of clinical factors and inflammatory biomarkers.[10] In the context of malaria, cortisol levels can rise as part of the body's stress response. However, the elevation of cortisol in malaria can be influenced by various factors, including the severity of the infection, immune response, and inflammatory processes.[11]

Majority of research on alterations in serum cortisol levels has focused on patients with P. falciparum malaria, given its global prevalence, rather than P. vivax malaria patients. However, considering the substantial number of P. vivax cases in India, the present study aimed to assess changes in serum cortisol levels among P. vivax malaria patients and correlate the levels with severity of the disease.

Objectives

1. To compare the Serum cortisol levels among uncomplicated and complicated adult plasmodium vivax patients.
2. To correlate the association between Serum cortisol levels with severity of the disease.

Materials and Methods

The current study was a prospective observational study done for a period of one year from September 2017 to August 2018. The study was done among Plasmodium Vivax malaria patients and healthy controls visiting the Department of Medicine, JLN medical College, Ajmer. Based on a mean difference of 4.55 and standard deviation of 1.43, the required sample size in each group was around 39.[12] Hence, A total of 120 patients were enrolled comprising of three groups of patients, out of which 40 are complicated P.vivax malaria patients, 40 were uncomplicated P. vivax malaria patients admitted in hospital and 40 were healthy controls. The controls were age and gender matched selected from the donors list in the blood bank.

Patients above 18 years of age in both genders, whose peripheral blood film was positive for plasmodium vivax malaria patients and age matched controls (blood donors in blood bank) were included. Patients who were known alcoholics, infections such as disseminated tuberculosis/ AIDS, prior use of drugs that diminish that diminish glucocorticoid production, such as glucocorticoids, megestrol, etomidate, or ketoconazole, Cushing's syndrome, Addison's disease, previous/present cardiorespiratory diseases, renal, hepatic diseases and pregnant patients were excluded.

The severity of the disease or its complications was defined in accordance with the World Health Organization's (WHO) guidelines for managing severe malaria in 2012. However, there was a modification for clinical jaundice/liver dysfunction, which means an increase in total bilirubin of ≥2.5 mg/dL along with a simultaneous three-fold elevation in any serum aminotransferases from their normal upper limits.[13] Severity determinants were restricted to cerebral malaria (impaired consciousness, coma or multiple generalized convulsions within 24 hours), liver dysfunction, pulmonary oedema (PE) or acute respiratory distress syndrome (ARDS) (radiological), renal failure (serum creatinine >3 mg/dL), shock (systolic blood pressure <80 mm Hg), spontaneous bleeding, hyperparasitaemia (parasite index >5%, i e, percentage of parasitized erythrocytes on Leishman-stained peripheral blood smear), hypoglycaemia (blood sugar <40 mg/dL), respiratory distress (respiratory rate >32 beats/minute), metabolic acidosis (plasma bicarbonate <15 mmol/L) and severe anaemia (haemoglobin <7 g/dL).[14]

Before enrolment, details about nature and utility of present study were explained to all patients and informed consent was taken. After enrolment all patients were subjected to a brief profiling, clinical examination and blood investigations. Details on their age, gender, clinical symptoms and signs were recorded in a data sheets. The patients were then subjected to recording of the vitals, blood investigations such as complete blood count, liver function test, renal function test and serum cortisol level was assed.

Serum sample of patients required for assay was collected between 7 to 9 a.m. on day 1 and day 7 of investigation and processed under aseptic conditions. Collected serum sample was allowed to clot adequately before centrifugation and ensured that all samples were free from fibrin, particulate matter or bubbles. Competitive immunoassay-based cortisol assay working on principal of competing sample cortisol with acridinium ester labeled cortisol in the Lite reagent for binding to polyclonal rabbit anti-cortisol antibody in the solid phase was performed using direct chemiluminescent technology.

Samples were treated by automatic system with following steps; 2 ml of sample was dispensed into a cuvette, 5 ml of Lite reagent and 25 ml of solid phase were then added sequentially and incubated for 5 minutes at 37°C. The cuvette was then separated, aspirated and washed with reagent water and 30 ml of each acid reagent and base reagent

were dispensed to initiate the chemiluminescent reaction. Finally, the observations were reported according to the selected reference interval (serum 4-22 µg/dl).

Statistical Analysis

The data collected was entered twice in Epidata for minimizing errors and analysed using SPSS version 16. The variables were summarized as mean and standard deviation for continuous variables and frequency percentage for categorical variables. The comparison between the groups for the blood investigations were done using student t test for two groups and one-way ANOVA for three groups. ROC curve and sensitivity analysis was done to calculate the cut-off of serum cortisol and obtain its validity as a tool to predict severity of malaria. A p value of less than 0.05 was considered significant.

Results

Data was collected from 40 patients in each group viz, Complicated P. vivax malaria, Uncomplicated P. vivax malaria patients and healthy controls. The average age of the patients was 28.5 (5.2), 29.8 (4.7) and 28.2 (5.7) years among Complicated P. vivax malaria, Uncomplicated P. vivax malaria patients and healthy controls.

Majority of the patients were males. The demographic and baseline clinical characteristics were statistically insignificant and similar in all three groups. However, there were few clinical variations in the vital parameters. There was mild elevation of temperature, pulse rate, respiratory rate, bilirubin, blood urea and serum creatinine values. The average pulse rate was 106.3 (10.3), 101 (9.8) and 79 (6.5) beats/ min among the complicated, uncomplicated and control group.

Similarly, respiratory rate was 35 (8.1), 22 (3.6) and 15 (2.5) per minute; temperature was 101.3 (1.29), 100.1 (1.3) and 98.7 (0.37) degree Fahrenheit; total Bilirubin was 2.08 (1.77), 0.9 (0.08) and 0.86 (0.07) mg/dl; blood urea was 57 (9.7), 28 (8.3) and 23 (6.4) mg/dl; serum creatinine was 1.81 (2.08), 0.87 (0.12) and 0.57 (0.14) mg/dl among the complicated, uncomplicated and control group respectively. (Table 1) Among the complications, bleeding was among 7 patients, cerebral malaria was among patients 5 patients, jaundice was among 9 patients, renal failure was among 8 patients, ARDS was among 11 patients, metabolic acidosis was among 4 and severe anemia was among 9 patients. (Figure 1)The various clinical and biochemical parameters were Compared between complicated and uncomplicated patients at day 1. (Table 2) Independent t test was done to find significant parameters. Comparison revealed significant lower hemoglobin and platelet values in the complicated group.

Additionally, the total bilirubin, blood urea and serum creatinine was significantly higher among the complicated group on comparison with uncomplicated group. Similarly, the serum cortisol levels were significantly higher in the complicated group on comparison with uncomplicated group [20.53 (4.3) vs15.3 (1.69)] The various clinical and biochemical parameters were Compared between complicated and uncomplicated patients at day 7. (Table 3)

However, all the parameters were similar in both the groups on day 7 except for lower hemoglobin level in the complicated group. The serum cortisol level was also significantly higher in the complicated group on comparison with uncomplicated group [15.49 (2.43) vs 13.89 (1.49)]. The parameters were also compared between the study group which includes both complicated and uncomplicated malaria patients and the control group. (Table 4) The comparison revealed significantly lower hemoglobin and platelet values in the study group. Additionally, temperature, total bilirubin and blood urea was significantly higher among the complicated group on comparison with uncomplicated group. The serum cortisol level was also significantly higher in the study group on comparison with control group [17.72 (4.12) vs 13.90 (1.71)] ROC analysis was done to find the optimum cut-off of serum cortisol to predict severity of malaria. Between the complicated and uncomplicated group, the optimum was 18.6 ug/dl for a sensitivity of 88.5% and specificity of 85.8%. The Area under the Curve (AUC) for the ROC curve was 0.934 (95% CI: 0.864- 0.961). The p value for the curve was less than 0.001.

Table 1: Comparison of Baseline demographic and clinical characteristics among the three groups on Day 1 (n=120)

Characteristics, mean (SD)	Complicated P. vivax Malaria (n=40)	uncomplicated P. vivax malaria patients (n=40)	Health controls (n=40)	p value*
Age (years)	28.5 (5.2)	29.8 (4.7)	28.2 (5.7)	0.629
Gender- Male, n(%)	28 (70%)	25 (62.5%)	27 (67.5%)	0.736
Pulse rate (/min)	106.3 (10.3)	101 (9.8)	79 (6.5)	0.577
Respiratory rate (/min)	35 (8.1)	22 (3.6)	15 (2.5)	0.075
Systolic Blood pressure (mmHg)	117.6 (5.1)	119.4 (4.9)	120.3 (4.1)	0.811
Diastolic Blood pressure	78.5 (3.9)	81.3 (5.3)	83.4 (3.8)	0.694

(mmHg)				
Temperature (°F)	101.3 (1.29)	100.1 (1.3)	98.7 (0.37)	0.356
Total Bilirubin (mg/dL)	2.08 (1.77)	0.9 (0.08)	0.86 (0.07)	0.390
Blood urea (mg/dL)	57 (9.7)	28 (8.3)	23 (6.4)	0.210
Serum Creatinine (mg/dL)	1.81 (2.08)	0.87 (0.12)	0.57 (0.14)	0.103

*p value was by independent t test; #-p<0.05

Table 2: Comparison between complicated and uncomplicated patients at day 1 in Study Group (n=80)

Parameters	Complicated	Uncomplicated	P value
	Mean (SD)	Mean (SD)	
Systolic BP (mmHg)	117.6 (5.1)	119.4 (4.9)	0.812
Diastolic BP (mmHg)	78.5 (3.9)	81.3 (5.3)	0.769
Temperature °F	101.3 (1.29)	100.1 (1.3)	0.311
Haemoglobin (gm%)	10.09 (1.5)	12.93 (0.72)	0.023
Platelet Count (lacs)	0.91 (0.41)	1.84 (0.28)	<0.001
Total Bilirubin (mg/dL)	2.08 (1.77)	0.9 (0.08)	0.001
Blood urea (mg/dL)	57 (9.7)	28 (8.3)	<0.001
Serum Creatinine (mg/dL)	1.81 (2.08)	0.87 (0.12)	<0.001
Cortisol Level (Ug/d)	20.53 (4.3)	15.3 (1.69)	<0.001

*p value was by independent t test; #-p<0.05

Table 3: Comparison between complicated and uncomplicated patients at day 7 in Study Group (n=80)

Parameters	Complicated	Uncomplicated	P value*
	Mean (SD)	Mean (SD)	
Systolic BP (mmHg)	118.7 (5.3)	117.6 (4.7)	0.961
Diastolic BP (mmHg)	79.3 (4.2)	80.8 (4.7)	0.745
Temperature °F	99.4 (1.23)	99.1 (0.4)	0.837
Haemoglobin (gm%)	12.99 (1.07)	14.93 (2.13)	<0.001
Platelet Count (lacs)	2.83 (0.64)	2.78 (0.52)	0.956
Total Bilirubin (mg/dL)	0.93 (1.77)	0.9 (0.08)	0.949
Blood urea (mg/dL)	31 (9.7)	23 (8.1)	0.189
Serum Creatinine (mg/dL)	0.89 (0.08)	0.82 (0.07)	0.968
Cortisol Level (Ug/d)	15.49 (2.43)	13.89 (1.49)	<0.001

*p value was by independent t test; #-p<0.05

Table 4: Comparison between Study and Control Groups on day 1 (n=120)

Parameters	Control Group	Study Group	P value*
	Mean (SD)	Mean (SD)	
Systolic BP (mmHg)	118.87 (4.32)	119.34 (4.41)	0.620
Diastolic BP (mmHg)	77.60 (4.15)	77.57 (3.74)	0.973
Temperature °F	98.53 (0.27)	100.36 (1.18)	<0.001
Haemoglobin (gm%)	14.66 (0.62)	12.24 (1.93)	<0.001
Platelet Count (lacs)	2.92 (0.60)	1.40 (0.60)	<0.001
Total Bilirubin	0.88 (0.07)	1.47 (1.50)	0.036
Serum Creatinine	0.92 (0.10)	1.37 (1.62)	0.129
Cortisol Level	13.90 (1.71)	17.72 (4.12)	<0.001

*p value was by independent t test; #-p<0.05

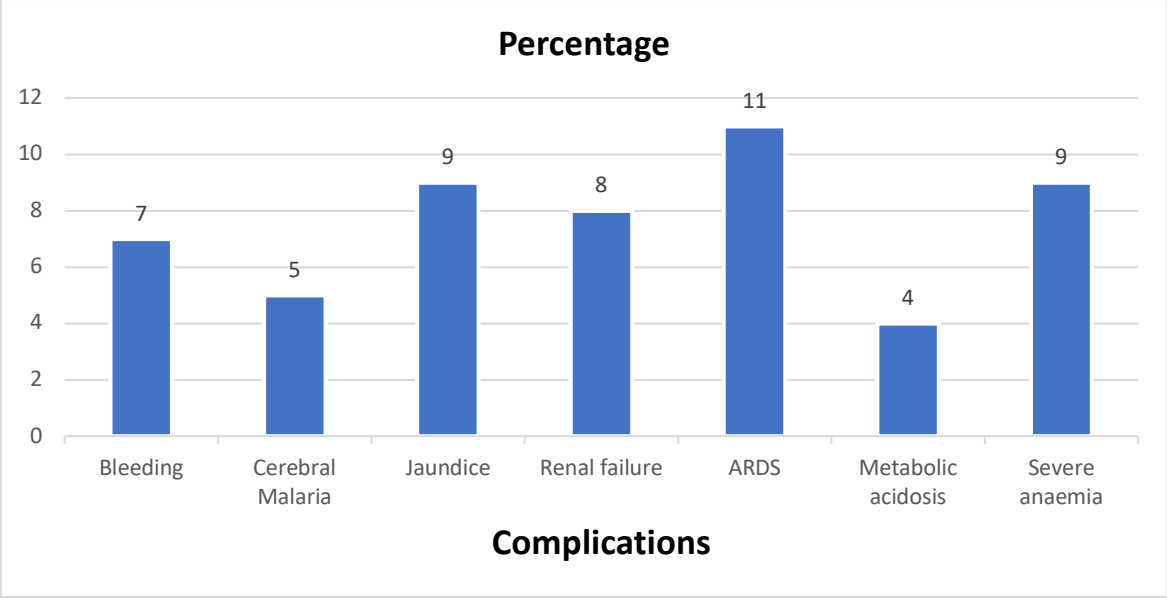

Figure 1: Distribution of complications among the P. vivax malaria patients (n=40)

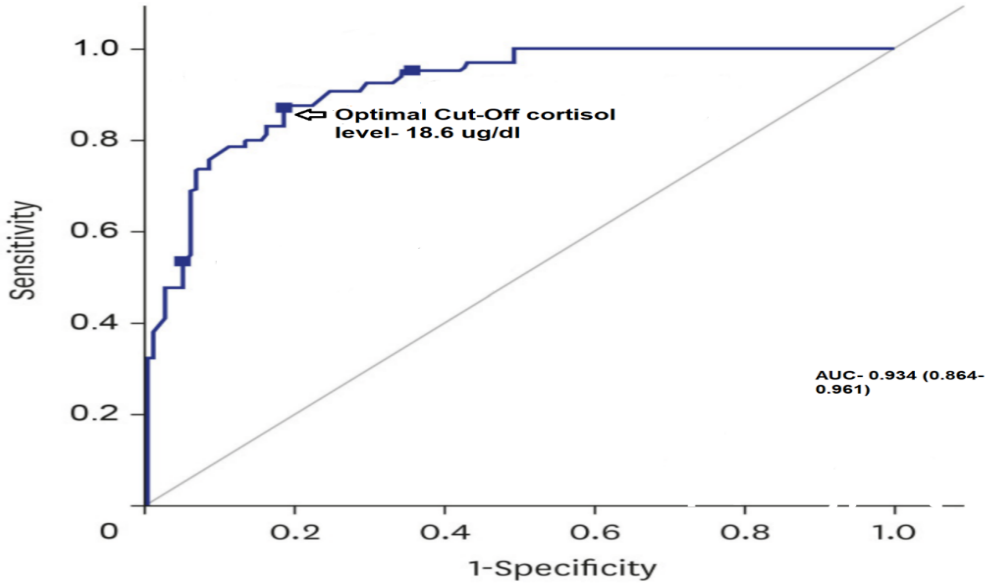

Figure 2: ROC curve for optimum cut-off cortisol level (n=80)

Discussion

Malaria is widespread in many regions of India, and in recent years, there has been a resurgence of malaria cases. Several factors have contributed to this resurgence, including the resistance of mosquitoes to insecticides, an increased prevalence of chloroquine-resistant malaria, and a rise in cases of falciparum malaria compared to vivax malaria. The desert area of Rajasthan, particularly the Ajmer district, has traditionally been considered hypo endemic for malaria, with only certain pockets showing the presence of P. falciparum. However, the last decade has seen a significant increase in developmental activities in this region, which, combined with global climatic changes, has led to marked changes in the epidemiological and clinical profile of malaria. The implementation of the Indira Gandhi Canal Project has resulted in increased irrigation activities, providing breeding grounds for mosquitoes. Moreover, there has been a notable influx of labourers from the Eastern part of the country, bringing with them potential malaria infections. Additionally, the massive deployment of armed forces in the area has likely played a role in the spread of the disease. Finally, the occurrence of unprecedented rains has further contributed to the changes in malaria dynamics in this region.

In the current study, the serum cortisol levels were significantly higher in the complicated group on comparison with uncomplicated group at both day 1 and day 7. Serum cortisol level was also significantly higher in the P. vivax malaria group

versus the healthy control group. Several studies have investigated the levels of serum cortisol in patients with malaria to understand the impact on the hypothalamic-pituitary-adrenocortical axis. Davis et al. (1997) assessed nine Vietnamese adults with complicated malaria and found that some patients exhibited primary and secondary adrenal insufficiency, potentially influenced by increased interleukin-6 levels and impaired cortisol metabolism.[15]

Shwe et al. (1998) conducted a study on patients with uncomplicated and cerebral malaria, along with controls. They observed elevated serum cortisol levels in both malaria groups compared to controls upon admission to the hospital, but no significant difference between uncomplicated and cerebral malaria patients.[16] Wilson et al. (2001) investigated pituitary-adrenal function in Vietnamese adults with acute uncomplicated falciparum malaria. They observed higher serum cortisol levels in patients before dexamethasone administration, and a slower overnight fall in serum cortisol compared to controls. The authors suggested a raised cortisol set point for ACTH inhibition but normal responsiveness to dexamethasone in uncomplicated malaria.[17]

Libonati et al. (2006) studied 24 patients with uncomplicated P. falciparum malaria in Brazil and found significantly higher cortisol levels on Day 0 compared to Day 7, indicating stimulation of the hypothalamic-pituitary-adrenal axis in these patients.[18] Ibrahim et al. (2011) investigated serum cortisol levels in patients with uncomplicated P. falciparum malaria in Sudan. They did not find a significant difference in cortisol levels between patients and controls.

However, cortisol levels were higher in patients on presentation compared to levels on day 7. Overall, these studies provide valuable insights into the relationship between malaria and cortisol levels, suggesting that malaria can influence the hypothalamic-pituitary-adrenocortical axis, leading to variations in cortisol responses.[19]

The current study had several limitations. Firstly, the small sample size of the study group was insufficient to draw definitive recommendations. Conducting follow-up investigations over a longer duration could have helped establish more meaningful correlations between the investigated parameter and the disease. Furthermore, the study did not explore primary and secondary adrenal insufficiency or other endocrinal manifestations that may occur in malaria patients. These aspects could be subjects for further research. In addition, the study did not examine the correlation between serum cortisol levels, temperature, parasitemia, and cytokines (specifically IL-6). Exploring these relationships could provide significant justification for the title and findings of the current study.

Conclusion

The severity of adult malaria can vary widely among different populations with varying levels of endemicity. Given the higher prevalence of P. vivax and a considerable proportion of broad-spectrum severity observed in this and previous studies, it is crucial to remain vigilant for potential impacts of P. vivax in addition to P. falciparum. The research demonstrated that serum cortisol levels were notably higher in patients with complicated P. vivax malaria compared to those with uncomplicated P. vivax malaria. Therefore, serum cortisol level could serve as a significant biomarker for assessing disease severity in adult patients with Plasmodium vivax and can be done routinely in the clinical settings. Additionally, the study identified an optimal cortisol cut-off value of 18.6 ug/dl that can be used to predict the severity of the patients' condition.

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