Original Research Article

Study of serum cortisol levels in complicated and uncomplicated *Plasmodium vivax* malaria patients

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ABSTRACT

Background: Malaria results in pathological changes in various body organs, as the parasite invade and multiply in circulating red blood cells. Despite of advances in diagnostic and treatment modalities, worldwide incidences of malaria are significant. Current study was conducted to investigate serum cortisol level changes as a promising biomarker for risk prediction in malaria and to study adrenal insufficiency in malaria patients.
Methods: Current investigation was a prospective observational study, conducted on complicated and uncomplicated Plasmodium vivax malaria patients. Serum cortisol levels in patients were investigated through immunoassay using direct chemiluminescent technology and were statistically correlated with Plasmodium vivax malaria infection.
Results: Results of present investigation revealed that on day 1 there was significant difference in mean serum cortisol levels between the *Plasmodium vivax* malaria patients and control group and cortisol levels were significantly higher in complicated *Plasmodium vivax* malaria patients compared to uncomplicated cases on day 1 and 7. Cortisol levels were observed to be normal on day 1 and 7 in uncomplicated malaria cases and in patients with bleeding manifestations, renal failure and jaundice. In 10 out of 15 cases of cerebral malaria, significant increase in serum cortisol levels were observed on day 1, while on day 7 levels were normal in all 15 cases.
Conclusions: Rise in serum cortisol level had a positive correlation with temperature and thus can be useful to predict the severity of disease in *Plasmodium vivax* malaria patients. No cortisol insufficiency was observed in during active and convalescent stages of illness.

Keywords: Malaria, Parasitic infection, *Plasmodium vivax*, *Plasmodium falciparum*, Serum cortisol

INTRODUCTION

Malaria is a parasitic infection caused by intraerythrocytic protozoa of genus Plasmodium. Malaria is primarily transmitted by the bite of an infected female anopheles mosquito.[1] Out of 172 subspecies of genus Plasmodium, that has an intracellular parasite accumulating malaria pigment, five species namely; *Plasmodium malariae*, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium knowlesi* can infect humans to cause malaria.[1] It was observed that *Plasmodium falciparum* is mostly responsible for the severe and fatal malaria, but recent published literature reports *Plasmodium vivax*

monoinfections as more fatal.[2] In 2017 malaria affected 219 million people globally, resulting in 435,000 deaths.[3] Malaria is considered as a major health threat specifically in tropical and subtropical regions across the world; mortality rate due to malaria ranges from 0.3-2.2% globally whereas the mortality rate is as high as 11-30% in tropical regions.[2,3] Malaria is a major cause of morbidity in Indian subcontinent also, with 95.5% of population at risk and 13-15% of total mortality rate due to malaria in the country.[4,5] Malaria can be a simple febrile illness or it may cause complications leading to critical illness. Common clinical features of uncomplicated malaria includes paroxysms of fever with associated symptoms like rigors, sweats, headache,

myalgia, back pain, abdominal pain, nausea, vomiting, diarrhea and pallor.[6] Clinical features of complicated/severe malaria include cerebral malaria, acidosis, normocytic normochromic anemia, renal failure, acute respiratory disease, hypoglycemia, hypotension, bleeding, hyperparasitemia and jaundice.[2,6] Malaria affects almost all organs of body including endocrine glands resulting in metabolic manifestations like hypovolemia, hypokalemia and hyperkalemia, hyponatremia, diabetes insipidus, hypocalcemia, hypoglycaemia, hyperglycemia and primary or secondary thyroid and adrenal insufficiencies.[1,2,6]

Plasmodium life cycle is complex and occurs in two phases; sexual and asexual, the vector mosquitoes and the vertebrate hosts.[7] In vectors sexual phase of the parasite's life cycle occurs and asexual phase occurs in intermediate hosts, humans. The parasite, as sporozoite enters the human blood and eventually hepatocytes where the first phase of asexual development occurs.[7,8] The asexual erythrocytic cycle produces merozoites leading to rupture of erythrocytes. The asexual cycle usually continues until controlled by the immune response or chemotherapy or until the patient dies. After invading red blood cells, some merozoites differentiate into sexual gametocytes following ingestion by another female mosquito. They then mature to male and female gametes in the blood meal, this series of events is known as sexual phase.[7-10] The sexual process and meiotic division following fertilization allow genetic recombination to occur.8 Management of malaria is more prevalent through prevention than treatment; most common methods for preventing malaria is biological control of mosquitoes and use of insecticide-treated bed nets (ITNs), transgenic mosquitoes manipulated for resistance to malaria parasites is a newer approach explored for prevention of malaria. Conventional and new antimalarial agents are used for treatment of maleria, antimalarial vaccines are also being explored as a strategy for malaria management.[11-14] Conventional diagnosis techniques used for prognosis of malaria include microscopic examination of blood films (thin/thick) stained with Giemsa's, Field's stain or fluorescent stain. Quantitative buffy coat technique; ParaSight F test based on the immunological capture of the Plasmodiums histidine-rich protein in whole blood, OptiMal assay, which is antibody-based detection of parasite lactate dehydrogenase, PCR-based diagnostic tests, enzyme linked immunosorbent assay, serological methods, indirect fluorescent antibody tests and molecular are some newer strategies used for diagnosis of malaria but the all available techniques are either too tedious and time consuming, costly or specific to only one particular strain of Plasmodium and PCR-based diagnostic tests developed for diagnosis are more applicable to large-scale surveys than clinical diagnosis, thus it is required to explore newer approaches for diagnosis of malaria and to determine severity of disease.[15-18]

Cortisol is a glucocorticoid produced in the human body by the adrenal gland.[19] Adrenal cortisol production is regulated by adrenocorticotropic hormone (ACTH), which is synthesized by the pituitary gland in response to hypothalamic corticotropin-releasing hormone (CRH).[20] Cortisol is vitally important for maintenance of vascular tone, endothelial integrity, vascular permeability, and total body water distribution. It also potentiates the vasoconstrictor actions of both endogenous and exogenous catecholamines.[19-21] In humans, the amount of cortisol present in the blood undergoes diurnal variation; the level peaks in the early morning (approximately 8 a.m.) and reaches its lowest level at about midnight.[22] It has been observed that disease incidences of cold, infection, trauma, etc and other disturbances to homeostasis stimulate the hypothalamic pituitary- adrenal (HPA) axis, thereby affecting the normal cortisol levels in the body depending on the severity of the illness.[21,22] It has been reported and known that malaria affects almost all organs of body including the endocrine glands.[23] In the year 1997, Davis et al described that patients with malaria can have features of adrenal insufficiency leading to disturbances in the cortisol levels.[23] Thus the purpose of this study was to see the occurrence of serum cortisol level changes and the possibility of adrenal insufficiency in malaria patients and to correlate it with severity of the disease to investigate it as a promising biomarker for high risk prediction.

Aim and objectives

Most of the previous study reports on serum cortisol changes have been conducted in malaria caused by Plasmodium falciparum. The aim of the current study was to determine the serum cortisol levels in uncomplicated and complicated *Plasmodim vivax* infected malaria patients with the objective of investigating the correlation of serum cortisol levels with severity of malarial disease.

METHODS

Study design, duration and location

Current study was a prospective observational study conducted at the department of medicine, Sardar Patel medical college and associate group of hospitals, Bikaner from November 2012- to November 2013.

Study population

Total 100 patients were enrolled in the study and subjected to detailed clinical examination and relevant investigations. Out of 100 enrolled patients, 35 were complicated Plasmodium vivax malaria patients, 35 were uncomplicated Plasmodium vivax malaria patients and 30 patients were taken as control (blood donors from blood bank). Before enrolment details about nature and utility of present study was explained to all patients and informed consent was taken.

Inclusion criteria

The criteria for inclusion in the current study were; complicated *Plasmodium vivax* malaria patients exhibiting clinical features like, cerebral malaria: failure to localize or respond appropriately to noxious stimuli; coma persisting for>30 minutes after generalized convulsion, renal failure: urine output (24 hours) of <400 ml in adults or <12 ml/kg in children; serum creatinine level of >265 mol/l with no signs of improvement after rehydration, bleeding/disseminated intravascular coagulation: significant bleeding and hemorrhage from the gums, nose, and gastrointestinal tract and/or evidence of disseminated intravascular coagulation, jaundice: serum bilirubin level of >50 mmol/l combined with other vital-organs dysfunction and normochromic, normocytic anemia: hematocrit of<15% or hemoglobin level of<50 g/l with parasitemia level of>100,000 per litre. Along with the above-mentioned features complicated and uncomplicated *Plasmodium vivax* malaria patients of either sex with positive peripheral blood film, falling in the age group above 15 years, were included in the study. Controls in matching age range were selected and included in the study.

Exclusion criteria

Alchoholic patients, patients with infections like disseminated tuberculosis or AIDS, patients having prior history of usage of drugs that diminish glucocorticoid production, such as glucocorticoids, megestrol, etomidate, or ketoconazole, patients having Cushings syndrome or Addisons disease, patients with previous/present cardio, respiratory, renal or hepatic diseases and pregnant patients were excluded from the study.

Procedure

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).

RESULTS

Mean of different parameters observed at day 1 in control group of patients is depicted in (Table 1). Mean age was patients was observed to be 28.17±4.96 years, mean systolic blood pressure was 118.87±4.32 mmHg, mean diastolic blood pressure was 77.60±4.15 mmHg, mean temperature was 98.53±0.27°C, mean haemoglobin was 14.66±0.62 gm%, mean platelet count was 2.92±0.60 lacs, mean total bilirubin was 0.88±0.07 mg/dl, mean serum creatinine was 0.92±0.10 mg/dl and mean cortisol level was observed to be 13.90±1.71 µg/dl.

Table 1: Mean of different parameters at day 1 in control group.

Parameters	Mean	SD	SE
Age (years)	28.17	4.96	0.90
Systolic BP (mmHg)	118.87	4.32	0.79
Diastolic BP (mmHg)	77.60	4.15	0.76
Temperature (°C)	98.53	0.27	0.04
Haemoglobin (gm%)	14.66	0.62	0.11
Platelet count (lacs)	2.92	0.60	0.11
Total bilirubin (mg/dl)	0.88	0.07	0.01
Serum creatinine (mg/dl)	0.92	0.10	0.02
Cortisol level (µg/dl)	13.90	1.71	0.31

Table 2: Statistical analysis showing comparison different parameters at day 1 and day 7 in bleeding group.

Parameters	Day 1			Day 7			t	P value
	Mean	SD	SE	Mean	SD	SE		
Systolic BP (mmHg)	120.00	2.83	1.15	117.67	5.13	2.09	0.976	0.352
Diastolic BP (mmHg)	79.00	2.10	0.86	76.33	4.97	2.03	1.212	0.254
Temperature (°C)	99.90	0.38	0.16	98.73	0.23	0.10	6.348	<0.001
Haemoglobin (gm%)	7.13	0.96	0.39	11.63	0.65	0.27	9.517	<0.001
Platelet count (lacs)	0.18	0.08	0.03	3.10	0.58	0.24	12.287	<0.001
Total bilirubin (mg/dl)	1.33	0.07	0.03	0.89	0.08	0.03	9.900	<0.001
Serum creatinine (mg/dl)	0.94	0.15	0.06	0.89	0.09	0.04	0.740	0.476
Cortisol level (µg/dl)	17.59	2.70	1.10	15.14	2.08	0.85	1.761	0.109

Table 3: Statistical analysis showing comparison different parameters at day 1 and day 7 in cerebral malaria group.

Parameters	Day 1			Day 7			t	P value
	Mean	SD	SE	Mean	SD	SE		
Systolic BP (mmHg)	120.40	4.22	1.09	120.00	4.34	1.12	0.256	0.800
Diastolic BP (mmHg)	77.87	2.77	0.72	78.13	3.58	0.93	0.228	0.821
Temperature (°C)	101.84	1.59	0.41	98.57	0.31	0.08	7.803	<0.001
Haemoglobin (gm%)	13.51	1.01	0.26	13.99	0.85	0.22	1.410	0.169
Platelet count (lacs)	1.05	0.22	0.05	2.93	0.61	0.02	11.266	<0.001
Total bilirubin (mg/dl)	0.99	0.12	0.03	0.87	0.07	0.02	3.398	0.002
Serum creatinine (mg/dl)	0.88	0.10	0.03	0.89	0.11	0.03	0.390	0.700
Cortisol level (µg/dl)	23.33	4.72	1.22	16.44	2.76	0.71	4.887	<0.001

Table 4: Statistical analysis showing comparison different parameters at day 1 and day 7 in jaundice group.

Parameters	Day 1			Day 7			t	P value
	Mean	SD	SE	Mean	SD	SE		
Systolic BP (mmHg)	118.00	5.13	1.81	119.25	4.65	1.64	0.511	0.618
Diastolic BP (mmHg)	77.25	5.01	1.77	78.25	4.06	1.44	0.439	0.668
Temperature (°C)	100.48	0.64	0.23	98.44	0.22	0.08	8.505	<0.001
Haemoglobin (gm%)	10.46	0.41	0.15	12.88	0.61	0.22	9.245	<0.001
Platelet count (lacs)	1.03	0.38	0.13	2.99	0.89	0.31	5.713	<0.001
Total bilirubin (mg/dl)	5.43	1.35	0.48	0.90	0.08	0.03	9.441	<0.001
Serum creatinine (mg/dl)	0.94	0.22	0.08	0.95	0.07	0.03	0.169	0.868
Cortisol level (µg/dl)	18.99	1.41	0.50	16.21	1.92	0.68	3.296	0.005

Results of statistical analysis, comparing different parameters at day 1 and day 7 in bleeding group are depicted in (Table 2). The difference in parameters between day 1 and day 7 were observed to be highly significant for parameters like temperature, haemoglobin, platelet count and total bilirugin (p<0.001), while insignificant results were observed for parameters like systolic blood pressure, diastolic blood pressure, serum creatinine and cortisol levels (p>0.05).

Statistical analysis results, comparing different parameters at day 1 and day 7 in cerebral malaria group are shown in (Table 3). Highly significant difference was observed in parameters like temperature, platelet count and cortisol levels (p<0.001), significant difference was observed for parameters like total bilirubin (p<0.01), while difference in parameters like systolic, diastolic blood pressure, haemoglobin and serum creratinine was observed to be insignificant (p>0.05).

Statistical analysis of comparison between day 1 and day 7 parameters in patients belonging to jaundice group is shown in (Table 4). Highly significant differences were observed in parameters like temperature, hemoglobin, platelet count and total bilirubin (p<0.001), significant difference was found in parameter cortisol level (p<0.01), while insignificant differences were observed for parameters like systolic blood pressure, diastolic blood pressure and serum creatinine (p>0.05).

Statistical analysis results of comparison between day 1 and day 7 parameters in patients belonging to renal failure group are revealed in (Table 5). Highly significant differences were observed in parameters like temperature and serum creatinine (p<0.001), significant difference was found in parameters platelet count and cortisol levels (p<0.01) and insignificant differences were observed in parameters like systolic blood pressure, diastolic blood pressure, haemoglobin and total bilirubin (p>0.05).

Results of statistical analysis, comparing different parameters at day 1 and day 7 in uncomplicated group are revealed in (Table 6). Highly significant differences were observed in parameters like temperature, haemoglobin and platelet count (p<0.001), significant difference was observed for parameter cortisol level (p<0.01) and insignificant differences were found in parameters like systolic blood pressure, diastolic blood pressure, total bilirubin and serum creatinine (p>0.05).

Results of statistical analysis, showing comparison of parameters between complicated and uncomplicated patients at day 1 in study group are depicted in (Table 7). Highly significant differences were observed in parameters like temperature, platelet count and cortisol level (p<0.001), significant differences were found in parameters like haemoglobin, total bilirubin (p<0.01) and serum creatinine (p<0.05), while insignificant differences were found in parameters like systolic and diastolic blood pressure (p>0.05).

Results of statistical analysis, showing comparison of parameters between complicated and uncomplicated patients at day 7 in study group are depicted in (Table 8). Highly significant differences were observed in parameters like haemoglobin and cortisol levels (p<0.001), while all other parameters were observed to be statistically insignificant (p>0.05).

Table 5: Statistical analysis showing comparison different parameters at day 1 and day 7 in renal failure group.

Parameters	Day 1			Day 7			t	P value
	Mean	SD	SE	Mean	SD	SE		
Systolic BP (mmHg)	119.00	3.74	1.53	122.00	5.51	2.25	1.103	0.296
Diastolic BP (mmHg)	76.67	3.72	1.52	78.00	2.19	0.89	0.756	0.467
Temperature (°C)	100.10	0.60	0.25	98.40	0.26	0.11	6.336	<0.001
Haemoglobin (gm%)	12.80	1.12	0.46	13.47	0.75	0.31	1.212	0.253
Platelet count (lacs)	1.34	0.20	0.08	2.80	0.70	0.28	4.897	0.001
Total bilirubin (mg/dl)	0.93	0.13	0.05	0.92	0.07	0.03	0.083	0.935
Serum creatinine (mg/dl)	6.37	1.84	0.75	0.87	0.07	0.03	7.316	<0.001
Cortisol level (μg/dl)	17.12	1.67	0.68	13.99	1.03	0.42	3.895	0.003

Table 6: Statistical analysis showing comparison different parameters at day 1 and day 7 in uncomplicated group.

Parameters	Day 1			Day 7			t	P value
	Mean	SD	SE	Mean	SD	SE		
Systolic BP (mmHg)	119.14	4.74	0.80	119.83	4.91	0.83	0.595	0.554
Diastolic BP (mmHg)	77.43	4.10	0.69	77.54	3.63	0.61	0.123	0.902
Temperature (°C)	99.83	0.54	0.09	8.53	0.29	0.05	12.524	<0.001
Haemoglobin (gm%)	12.87	0.51	0.09	14.53	0.74	0.12	10.946	<0.001
Platelet count (lacs)	1.856	0.31	0.05	2.96	0.63	0.11	9.269	<0.001
Total bilirubin (mg/dl)	0.89	0.08	0.01	0.89	0.07	0.01	0.177	0.860
Serum creatinine (mg/dl)	0.90	0.09	0.02	0.90	0.08	0.01	0.109	0.913
Cortisol level (μg/dl)	15.15	1.49	0.25	13.98	1.55	0.26	3.208	0.002

Table 7: Statistical analysis showing comparison between complicated and uncomplicated patients at day 1 in study group.

Parameters	Complicated			Uncomplicated			t	P value
	Mean	SD	SE	Mean	SD	SE		
Systolic BP (mmHg)	119.54	4.12	0.70	119.14	4.74	0.80	0.377	0.707
Diastolic BP (mmHg)	77.71	3.40	0.57	77.43	4.10	0.69	0.317	0.752
Temperature (°C)	100.90	1.39	0.23	99.83	0.54	0.09	4.246	<0.001
Haemoglobin (gm%)	11.60	2.54	0.43	12.87	0.51	0.09	2.900	0.005
Platelet count (lacs)	0.94	0.44	0.07	1.86	0.31	0.05	9.948	<0.001
Total bilirubin (mg/dl)	2.05	1.97	0.33	0.87	0.08	0.01	3.495	0.001
Serum creatinine (mg/dl)	1.84	2.21	0.37	0.90	0.10	0.02	2.529	0.014
Cortisol level (μg/dl)	20.29	4.31	0.73	15.15	1.49	0.25	6.660	<0.001

Table 8: Statistical analysis showing comparison between complicated and uncomplicated patients at day 7 in study group.

Parameters	Complicated			Uncomplicated			t	P value
	Mean	SD	SE	Mean	SD	SE		
Systolic BP (mmHg)	119.77	4.72	0.80	119.83	4.91	0.83	0.050	0.961
Diastolic BP (mmHg)	77.83	3.67	0.62	77.54	3.63	0.61	0.327	0.745
Temperature (°C)	98.54	0.29	0.05	98.53	0.29	0.05	0.207	0.837
Haemoglobin (gm%)	13.24	1.12	0.19	14.53	0.74	0.12	5.669	<0.001
Platelet count (lacs)	2.95	0.66	0.11	2.96	0.63	0.11	0.056	0.956
Total bilirubin (mg/dl)	0.89	0.07	0.01	0.89	0.08	0.01	0.065	0.949
Serum creatinine (mg/dl)	0.90	0.09	0.02	0.90	0.08	0.01	0.040	0.968
Cortisol level (μg/dl)	15.74	2.36	0.40	13.98	1.55	0.26	3.690	<0.001

Table 9: Statistical analysis showing comparison between study and control groups on day 1.

Parameters	Control group			Study group			t	P value
	Mean	SD	SE	Mean	SD	SE		
Systolic BP (mmHg)	118.87	4.32	0.79	119.34	4.41	0.53	0.498	0.620
Diastolic BP (mmHg)	77.60	4.15	0.76	77.57	3.74	0.45	0.034	0.973
Temperature (°C)	98.53	0.27	0.05	100.36	1.18	0.14	8.438	<0.001
Haemoglobin (gm%)	14.66	0.62	0.11	12.24	1.93	0.23	6.706	<0.001
Platelet count (lacs)	2.92	0.60	0.11	1.40	0.60	0.07	11.704	<0.001
Total bilirubin (mg/dl)	0.88	0.07	0.14	1.47	1.500	0.18	2.126	0.036
Serum creatinine (mg/dl)	0.92	0.10	0.02	1.37	1.62	0.19	1.529	0.129
Cortisol level (µg/dl)	13.90	1.71	0.31	17.72	4.12	0.49	4.887	<0.001

Table 10: Distribution of cases according to cortisol levels in relation to complicated and uncomplicated cases.

Complication	Cortisol level (µg/dl)							
	Day 1				Day 7			
	≤22		>22		≤22		>22	
	N	%	N	%	N	%	N	%
Bleeding	6	100	0	-	6	100	0	-
Cerebral malaria	5	33.3	10	66.7	15	100	0	-
Jaundice	8	100	0	-	8	100	0	-
Renal failure	6	100	0	-	6	100	0	-
Uncomplicated	35	100	0	-	35	100	0	-
Total	60	85.7	10	14.3	70	100	0	-
Mean	16.30		26.25		14.86		0.00	
SD	2.24		1.69		2.17		-	
t	13.431				-			
P value	<0.001				-			

Results of statistical analysis, showing comparison between study and control groups parameters on day 1 are revealed in (Table 9). Highly significant differences were observed in parameters like temperature, haemoglobin, platelet count and cortisol level (p<0.001), significant difference was found in parameter total bilirubin (<0.05) and insignificant differences were observed for parameters like systolic, diastolic blood pressure and serum creatinine (p>0.05).

Distributions of cases according to cortisol level in relation to complicated and uncomplicated cases are revealed in (Table 10). On day 7 no patient had cortisol level>22 µg/dl, while on day 1, 10 patients had their cortisol level>22 µg/dl and they all belonged to cerebral malaria group. Difference in the mean cortisol levels between higher and lower ranges at day 1 and day 7 were statistically observed to highly significant (p<0.001) (Table 10).

DISCUSSION

Many parts of India are endemic to malaria; a parasitic infection transmitted by the female anopheles mosquito, resulting in clinical illness and pathological changes in various body organs due to invasion and multiplication of parasite in circulating red blood cells. In India, about 70% of the malarial infections are reported to be caused due to *Plasmodium vivax*, 25-30% due to *Plasmodium falciparum* and 4-8% cases are due to mixed infection.[24,25] In the present investigation 70 smear positive *Plasmodium vivax* malaria cases were studied out of which 35 were complicated (15 cerebral malaria, 6 bleeding, 8 jaundice, 6 renal failure) and 35 were uncomplicated malarial cases.

Results of present investigation revealed, highly significant differences in temperature, platelet count and serum cortisol levels and significant differences in haemoglobin, total bilirubin and serum creatinine levels between complicated and uncomplicated *Plasmodium vivax* malaria patients on day 1. Serum cortisol levels were observed to be significantly higher in all 70 malaria patients than in control group on day 1. Results of day 7 investigations of current study revealed significant differences in haemoglobin and cortisol levels, between complicated and uncomplicated patients, while all other parameters were found to be statistically insignificant. It was observed in current study that on day 1, among 70 cases, 10 cerebral malaria patients were found to have serum cortisol level>22 µg/dl (above normal range) with mean value of 26.25+1.69 µg/dl and the remaining 60 patients (5 cerebral malaria cases, 8 jaundice cases, 6 renal failure cases, 6 bleeding and 35 uncomplicated

Plasmodium vivax malaria cases) showed serum cortisol level <22 µg/dl (within normal range) with mean value of 16.30+2.24 µg/dl, while on day 7 all 70 patients were found to have serum cortisol level within normal range. This increase in serum cortisol level in cerebral malaria cases on day1 had a positive correlation with increased temperature on day 1.

Current investigation thus revealed that temperature interfered with the cortisol level suggesting stimulation of this hormone in malaria patients. It was also revealed in current study findings that no cortisol insufficiency in *Plasmodium vivax* malaria patients was observed during active and convalescent stages of illness. Some similar investigations are reported in published literature; Davis et al in their study assessed the hypothalamic-pituitary-adrenocortical axis in nine Vietnamese adults with complicated malaria.[23] Corticotropin releasing hormone (CRH) test was performed on patients (convalescence in five cases) and six healthy controls, basal plasma adrenocorticotropic hormone (ACTH) concentrations in malaria patients and controls were observed to be similar, while serum cortisol levels were found to be greater in three out of six patients compared to controls.[23]

It was thus observed that serum cortisol responses to CRH were depressed in acute illness, this concluded that, relative to a normal stress response, primary and secondary adrenal insufficiency can occur in severe malaria but may be attenuated by increased circulating interleukin-6 concentrations and impaired cortisol metabolism.[23] Wilson et al investigated pituitary-adrenal function in thirteen Vietnamese adults of acute uncomplicated falciparum malaria and 6 healthy controls and found higher serum cortisol levels in the patients than in the controls.[26] The report concluded that there was a raised set point for cortisol inhibition of ACTH secretion, but corticotrophin responsiveness to dexamethasone in uncomplicated malaria was observed to be normal.[26] In a similar study report conducted by Shwe et al on ten patients with uncomplicated malaria, ten with cerebral malaria and thirty-seven controls, mean serum cortisol level of patients with uncomplicated malaria was found to be 528.2±123.9 nmol/l, with cerebral malaria it was 516.0±80.5 nmol/L, and in controls it was 393.8±141.0 nmol/l, thus it was concluded from the investigation that significant rise in serum cortisol levels was observed in patients with malaria when compared to controls, the study group also reported that there was no cortisol insufficiency in cases with falciparum malaria during acute and convalescent stages of illness.[27] Libonati et al studied the behaviour of cortisol in 24 patients with uncomplicated *Plasmodium falciparum* malaria cases in Brazil, the report concluded that an increase in cortisol levels occurred in patients with *Plasmodium falciparum* malaria, indicating stimulation of the HPA axis in these patients.[28] Ibrahim et al investigated the levels of serum cortisol in patients with uncomplicated *Plasmodium falciparum* malaria in an area of unstable malaria transmission in Sudan and reported that there was no

significant difference in mean+SD of total cortisol levels in malaria patients when compared to control group.[29]

Limitations

Limitations of the current study were; the small sample size of the study group was not adequate to make concrete recommendations. Follow up investigations, for a longer duration could have aided to establish more significant correlations between the investigated parameter and the disease. Primary and secondary adrenal insufficiency and other endocrinal manifestations which can occur in malaria patients were not investigated in current study and can be further explored. Correlation between serum cortisol level, temperature, parasitemia and cytokines (IL- 6) can also be explored to significantly justify the current study title.

CONCLUSION

Plasmodium vivax infection has always been considered much less severe and usually not associated with any severe complications in comparison to *Plasmodium falciparum* infection. However, it has been observed that many patients with malaria due to *Plasmodium vivax* also develop complications like cerebral malaria, renal failure, jaundice and bleeding manifestations. It was observed and concluded through current investigation that serum cortisol level is increased in cerebral malaria cases caused due to *Plasmodium vivax*, during acute stage and this rise in serum cortisol level had a positive correlation with temperature which suggest stimulation of cortisol in malaria patients possibly due to hypothalamic pituitary-adrenal axis stimulation. Thus, it was inferred from current study findings that rise in serum cortisol level during acute stage can be useful to predict the severity of disease in *Plasmodium vivax* malaria patients. It was also concluded through current investigation that no cortisol insufficiency resulted in *Plasmodium vivax* malaria patients during active and convalescent stages of illness.

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