

SF Journal of Medicine and Research

Angiopoietin-1 and 2 in Falciparum Malaria: Its Dysregulation and Role as Biomarkers for Prediction of Outcome

Mohapatra MK*, Gnanasekaran GS, Mohanty PK and Bariha PK

Department of Medicine, V.S.S Institute of Medical Sciences and Research (VIMSAR), Burla, Odisha, India

Abstract

Angiopoietin-1 and 2 maintain the integrity of endothelium, which plays a pivotal role in the pathogenesis of severe malaria. We study its dysregulation and utility as biomarker.

We enrolled 34 patients of uncomplicated, 108 patients of severe falciparum malaria (SM), and 30 healthy controls in this study. Plasma ANG-1 and 2 were estimated with sandwich ELISA and analyzed for predictive accuracy.

There was a fall in ANG-1 ($p < 0.001$) and increase in ANG-2 ($p < 0.001$) levels and the ratio of ANG-2: ANG-1 ($p < 0.001$) in patients with SM. ROC analysis demonstrated that ANG-1, 2, and ratio of ANG-2 to 1 can discriminate between uncomplicated and SFM. It showed that plasma ANG-1, 2, and ratio are informative biomarkers of SFM.

Background: Adhesion of parasitized RBC and endothelial activation has been considered as an important pathogenic mechanism of severe falciparum malaria. Angiopoietin-1 and 2 play important role to maintain endothelial integrity. The objective is to study dysregulation of angiopoietins and their utility as biomarkers of disease severity in falciparum malaria.

Material and Methods: Enrolled patients were clinically characterized as healthy controls ($n=30$), uncomplicated (UM) ($n=34$) and severe falciparum malaria (SM) ($n=108$). In addition to detailed hematological and biochemical investigations, plasma ANG-1 and 2 were estimated with sandwich ELISA. ROC curve analysis was used to calculate area under the curve for each biomarker in order to assess predictive accuracy.

Results: Compared to UM there was significant decrease in ANG-1 ($p < 0.001$) and increase in ANG-2 ($p < 0.001$) levels and the ratio of ANG-2: ANG-1 ($p < 0.001$) in patients with SFM. There was a significant decrease in ANG-1 levels in patients with MODS, renal dysfunction, coma, and ARDS. Among patients with severe disease, ANG-2 levels correlated with MSS. ROC curve analysis demonstrated that the levels of ANG-1, 2, and ratio of ANG-2 to 1 discriminate between individuals with UM and SM.

Conclusion: These results showed that plasma ANG-1, 2, and ratio are informative biomarkers of disease severity in falciparum malaria.

Introduction

Malaria is a parasitic disease caused by five different species of Plasmodia, affecting about 500 million people of about 109 countries of the world [1]. Malaria due to *P. falciparum* is usually associated with various fatal complications leading to severe malaria resulting in high mortality [2]. Though Cerebral Malaria (CM) is the most common and fatal complication of in falciparum malaria, various non-cerebral complications like anemia, jaundice, renal failure etc. are also found along with cerebral malaria causing Multi Organ Dysfunctions (MODS) [3,4]. At this critical stage of the disease, patients require organ support at intensive care set up because of increased mortality.

While managing such cases we came across two important issues, first, to differentiate non-malarial acute febrile illnesses that masquerade CM and timely decision for critical care management to prevent progression of the disease and death. But no definite laboratory tests are available for definitive diagnosis of CM resulting in misdiagnosis and adverse outcome. Therefore, there is a need for a reliable biomarker that could not only distinguish CM from other causes, but also prognosticate and identify patients of severe malaria (with or without CM) who are at high risk

OPEN ACCESS

*Correspondence:

Manoj Kumar Mohapatra, Department of Medicine, V.S.S Institute of Medical Sciences and Research (VIMSAR), Burla, Sambalpur, Odisha, India.

E-mail: mohapatra.manoj@rediffmail.com

Received Date: 12 Jul 2020

Accepted Date: 04 Sep 2020

Published Date: 10 Sep 2020

Citation: Mohapatra MK, Gnanasekaran GS, Mohanty PK, Bariha PK. Angiopoietin-1 and 2 in Falciparum Malaria: Its Dysregulation and Role as Biomarkers for Prediction of Outcome. SF J Med Res. 2020; 1(3): 1014.

Copyright © 2020 Mohapatra MK. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

of mortality for intensified care treatment. In this connection many biological molecules have been tried but endothelial biomarkers are considered more physiological because endothelial activation plays a pivotal role in the pathogenesis of severe falciparum malaria (SM) including CM [5].

The integrity of endothelium is maintained by a distinct family of angiogenic proteins known as angiopoietins. In healthy state, the endothelium is quiet without any activation and this quiescence is maintained by Angiopoietin-1 (ANG-1) the level of which is higher than Angiopoietin-2 (ANG-2). Exogenous stimuli release ANG-2 that damages the endothelium by destabilizing its quiescent stage to an activated responsive endothelium [6].

Recent studies on experimental and human CM showed that endothelial activation promotes vascular permeability and damage of the Blood Brain Barrier (BBB) resulting in leakage of plasma proteins, perivascular edema, micro hemorrhages, and neuronal injury [7]. Therefore, we have undertaken this study to investigate dysregulation of Ang-1 and 2 in SFM and to evaluate its utility in prognosis and discrimination of fatal cases.

Material and Methods

The present study was conducted at VSS Medical College, Burla now renamed as V.S.S Institute of Medical Science and Research, Burla, Sambalpur, Odisha, India, which is situated between the geographical coordinates of 21.5°N 83.87°E from October 2012 to September 2018.

After getting approval from Institutional Ethics Committee (IEC) adult patients (>14 years of age) with falciparum malaria were enrolled in this study.

The diagnosis of falciparum malaria was made either with detection of asexual form of the parasite in the Giemsa stained peripheral blood smears or through Rapid Diagnostic Test. The parasitic count per μL was calculated from peripheral blood smear by multiplying number of asexual form of parasites per 200 leukocytes with total leukocyte count. Smear was taken daily to know the parasite clearance with treatment. CM and other forms of SM were defined according to the WHO criteria [2]. As the severity of organ dysfunction cannot be assessed appropriately from the clinical diagnosis of SM, we developed Malaria Severity Score (MSS) as an objective tool to assess the severity that ranged from 1 to 21 [8].

At the time of enrolment, demographic information and clinical features were recorded. Blood was collected for biochemical investigations required for diagnosis of organ dysfunction and base line data at the time of admission or within 12 hours of admission.

The investigations included blood glucose, blood urea, serum creatinine, serum sodium, serum albumin, serum bilirubin, Aspartate amino Transferase (AST), Alanine amino Transferase (ALT) and Complete Blood Count (CBC).

Cerebrospinal fluid analysis, abdominal ultrasound, chest X-ray, electrocardiogram, and serological markers for hepatitis were performed to exclude the diseases mentioned in exclusion criteria.

Plasma ANG-1 and ANG-2 levels were assessed by sandwich ELISA according to the manufacture's instruction at the laboratory of RMRC, ICMR, Bhubaneswar [9,10]. The lower and upper limit of detection for ANG-1 and ANG-2 were 156.25 to 10,000 pg/mL (0.15 to 10.0 ng/mL) and 54.69 to 3500.0 pg/mL (0.05 to 3.5 ng/mL).

Table 1: Baseline characteristics of patients of SFM.

Variables	SFM (Survivors) N=98	SFM (Death) N=10	P value
Male/ Female	60/38	7/3	-
Age (Years)	36.5±14.0	37.7±16.2	<0.1
Duration of coma (hour)	48.4±22.3	80.0±28.1	<0.001
Glasgow Coma Scale (GCS) score	11.5±3.1	7.5±2.6	<0.006
Heart rate (No. of beats/minute)	97.9±10.6	111.4±10.4	<0.001
Mean blood pressure (mmHg)	104.2±5.2	85.1±15.1	<0.001
Temperature (°F)	101.7±1.8	103.1±2.8	<0.002
Respiration rate (No./minute)	23.2±7.1	35.3±5.6	<0.001
Urine output (ml/24 hour)	1020.0±353.2	846.1±388.0	<0.001
Blood glucose (mg/dL)	74.2±16.1	72.4±64.5	0.217
S. bilirubin (mg/dL)	4.6±4.3	7.9±9.2	<0.001
S. albumin (gm/dL)	5.1±0.7	4.3±1.1	<0.01
Blood Urea (mg/dL)	54.6±37.6	141.3±50.3	<0.001
S. Creatinine (mg/dL)	4.3±1.2	8.7±1.4	<0.001
S. sodium (mEq/L)	132.6±5.2	129.1±25.4	<0.001
Hematocrit (%)	31.5±5.3	33.0±7.1	<0.002
Hemoglobin (gm/dL)	8.8±2.4	6.8±2.2	<0.001
Total leukocyte count (thousand/ μL)	8332.5±3561.5	8965.3±3328.6	<0.001
Platelet count ($1 \times 10^9/\mu\text{L}$)	2.0±1.6	1.4±0.6	<0.001
Parasitic count (thousand/ μL)	4123.9±1789.4	4232.9±1899.4	0.72
Malaria Severity Score (MSS)	6.7±1.1	11.2±4.1	<0.001

Detection in our samples diluted in 1:4 ranged from 71.9 to 7520.6 pg/ml for Ang-1 and 11.6 to 3500.0 pg/ml for Ang-2.

All patients were treated with injection Artesunate 2.4 mg/kg at 0 hrs., 12 hrs., 24 hrs. then once daily till they were able to tolerate drugs orally according to WHO guidelines along with organ supportive treatment [11]. Then artesunate and sulfadoxine-pyrimethamine was given orally.

Patients with co-morbid conditions like diabetes mellitus, chronic renal failure, hypertension, coronary artery disease, valvular heart disease, chronic liver disease, alcoholic liver disease, associated infections (e.g., pneumonia, urinary tract infection, viral hepatitis, meningitis), and who lost follow up were excluded from the study.

The statistical analysis was performed using IBM SPSS version 20. Comparative statistics were performed from a non-parametric univariate analysis using Mann-Whitney U test or Chi-square test as appropriate. Receiver operating characteristic curves and the area under the curves were generated using the IBM SPSS version 20 statistical software.

Results

We enrolled 142 patients of *P. falciparum* malaria and 30 age and sex matched healthy controls for the study. There were 34 (23.9%) patients of uncomplicated falciparum malaria (UM) and 108 (76.1%) patients of severe falciparum malaria (SM). Of 108 study participants with SM, 58 (53.7%) patients had Single Organ Dysfunction (SOD) and 50 (46.3%) patients with MODS.

Among 58 patients with SOD, cerebral malaria, jaundice, Acute Respiratory Distress Syndrome (ARDS), and anemia was

Table 2: Angiotensin level in different groups.

Variables	Healthy controls (A)	UFM (B)	SFM (C)	P value: A vs B/ B vs C
Number	30	34	108	
ANG 1 (ng/ml)	8.9±1.2	4.7±0.24	2.3±1.2	<0.001/<0.001
ANG 2 (ng/ml)	0.52±0.8	1.1±0.11	7.87±3.38	<0.001/<0.001
Ratio (ANG 2/ANG 1)	0.05±0.1	0.43±0.25	3.34±2.74	<0.001/<0.001

Table 3: Angiotensin level in different types of SFM.

Types of Malaria	Number (%)	ANG-1 (ng/ml)	ANG-2 (ng/ml)	ANG2:1 (Ratio)
A.CM+MOD (All)	50	2.4±1.1	9.4±3.2	4.7±3.1
A.i) CM+RF+J+RD	16(32.0%)	1.9±0.8	12.2±2.4	7.2±3.9
A.ii) CM+RF+J	24(48.0%)	2.5±1.2	9.9±2.2	3.6±1.2
A.iii) CM+A+J	10(20.0%)	2.8±0.4	7.4±2.5	3.5±2.1
B. SOD (All)	58	3.5±1.2	6.5±2.9	2.1±1.4
B.i) CM	12(20.7%)	2.1±0.7	9.7±2.3	4.7±1.7
B.ii) RD	10(17.2%)	3.3±0.5	7.4±2.5	2.1±0.5
B.iii) Jaundice	20(34.5%)	3.4±0.4	4.8±0.7	1.5±0.3
B.iv) Anaemia	16 (17.2%)	4.1±1.8	5.1±1.2	1.3±0.4
P value	-	A vs B<0.01 A vs A.i, A.ii<0.01 A vs A.iii, A.iv>0.5 B vs Bi,Bii>0.5 B vs Biii<0.01	A vs B<0.01 A vs A.i, A.ii, A.iv<0.01 A vs A.iii>0.5 B vs B.i>0.5 B vs Bii, Biii<0.01	A vs B<0.01 A vs A.i>0.5 A vs A.ii,A.iii,A.iv< 0.001 B vs B.i>0.5 B vs Bii, Biii<0.001

present in 12 (20.7%), 20 (34.5%), 10 (17.2%), and 16 (27.6%) cases, respectively. Out of MODS 2 organ dysfunction (CM+jaundice), 3 organ dysfunction (CM+jaundice+renal failure; RF) and 4 organ dysfunctions (CM+jaundice+RF+ARDS) was present in 10 (20.0%), 24 (48.0%), and 16 (32.0%) patients respectively. The mean MSS was 11.8±3.5 among patients with MODS and 2.7±1.1 among SOD ($p<0.001$). The MSS among survivors was 6.7±2.1 and among death was 11.2±4.1 ($p<0.001$). The base line characteristics and laboratory values were given in Table 1.

The mean ANG-1 level was 8.9±1.2 ng/ml in healthy controls and was about 2 fold (4.7±0.2 ng/ml) low among UM ($p<0.01$) and 4 fold (2.3±1.2 ng/ml) low among all patients with SM irrespective of complications and severity ($p<0.001$). Among the patients of MODS, ANG-1 (2.4±1.1 ng/ml) was lower than those with SOD (3.5±1.2 ng/ml) ($p<0.01$). However, the decrease in ANG-1 among patients with SOD is less compared to MOD. Comparison among each group showed that patients with 4 organ dysfunction (CM+RF+jaundice+ARDS) the level of ANG-1 (1.9±0.8 ng/ml) is significantly lower than mean value of MODS and other groups ($p<0.001$). Among the patients of SOD, patients with CM, RF, and ARDS had lower values of ANG-1 whereas patients with jaundice and anemia had higher level ANG-1 than CM (Table 2 and 3).

ANG-2 level increased from 0.52±0.8 ng/ml among healthy controls to 1.1±0.1 ng/ml among UM ($p<0.001$) and 7.8±3.3 ng/ml among SM ($p<0.001$).

ANG-2 level is progressively increased with disease severity and in different complications compared to healthy controls and UM. The median ratio of ANG-2/ANG-1 also progressively increased with diseases severity. However, ANG-1 level decreases in SFM in all groups taken together. Though among all MOD taken together it decreases 4-fold, the decrease in CM+RF+jaundice+ARDS is less compared to MOD whereas among 2 and 3 organ dysfunction it is comparable to

Table 4: Area under the curve.

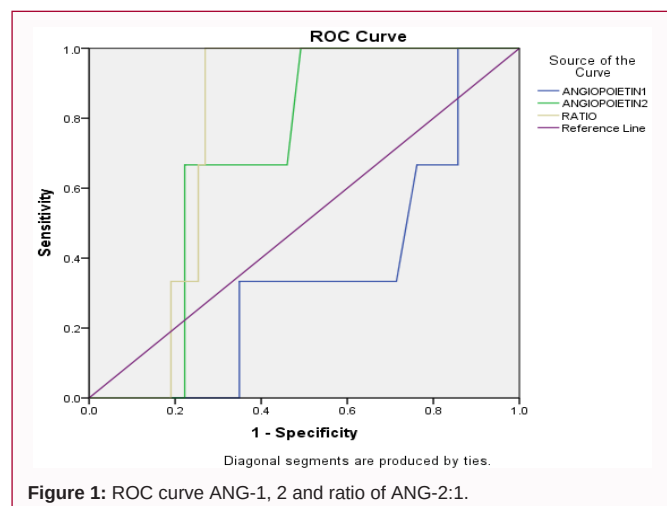
Test Result Variable(s)	Area
ANG-1	0.352
ANG-2	0.693
Ratio	0.762

CM. In other words, the decrease in 4 organ dysfunctions is more than other organs. Similarly, the fall in SOD though more than CM, but not in a progressive manner as it is found in rise of ANG-2 and ANG-2/ANG-1 ratio.

Ratio of ANG-2: ANG-1 holds significance because of their divergent role in vascular activation. Therefore, the ratio of ANG-2 to ANG-1 was taken as a parameter and the ratio was significantly higher in complicated malaria (3.3±2.7) compared to uncomplicated malaria (0.4±0.2) ($p<0.001$) and control (0.05±0.1) ($p<0.001$).

Out of 108 patients with SM, 10 (9.3%) patients died. The mean ANG-1, 2, and ratio among survivors are 2.8±1.8 ng/ml, 4.1±2.8 ng/ml, 3.4±1.1 and non-survivors are 1.7±1.2 ng/ml ($p<0.01$), 6.5±1.8 ng/ml ($p<0.01$), 5.2±1.1 ($p<0.01$) respectively. Lower ANG-1 level, higher ANG-2 level, and increased ratio of ANG-2/ANG-1 was associated with high mortality.

We plotted Receiver Operating Characteristic (ROC) curves ANG-1, ANG- 2 and ANG-2: ANG-1 ratio as a biomarker of severity using MSS as the standard to classify the severity. Each was assessed for their ability to discriminate between complicated and uncomplicated malaria. The Area Under the Curve (AUC) (Table 4) for ANG -1, ANG- 2 and their ratio were found to be 0.352, 0.693 and 0.762 respectively. This shows that ANG-2/ANG-1 ratio and ANG-2 concentration can discriminate patients of complicated malaria from uncomplicated malaria compared to ANG-1. The ANG-2/ANG-1 at a cut-off ratio of 0.80 is 86% sensitive and 81% specific. ANG-2 at a cut-



off value of value of 1.8 ng/ml has sensitivity of 90.7% and specificity of 89.5%. ANG-1 concentration at a cut-off value of 2.93 ng/ml has sensitivity of 85.7% and specificity of 52.5% (Figure 1).

Discussion

Rosette formation, cytoadherence, and sequestration of pRBC giving rise to hypoxia and organ damage has been ascribed as the main pathogenetic mechanisms of SM and CM. However, experimental, and human CM showed that endothelial activation plays a pivotal role in CM.

Anatomically, endothelium represents the boundary between vascular space and vital organs and in brain it is an integral part of Blood-Brain-Barrier (BBB). Under normal physiological condition, the state of normalcy is maintained by angiopoietin-Tie2 system. ANG-1 is released from perivascular cells including pericytes and smooth muscle cells and signals through the Tie-2 receptors to maintain vascular quiescence and integrity keeping tight junctions intact [12]. With exogenous stimulus, the endothelial cells release ANG-2 from Weibel-Palade (WP) bodies which inhibits signaling of ANG-1 and antagonizes its function, resulting in endothelial cell activation, augmentation of inflammation, loosening of tight junctions and increasing BBB permeability. The evidences in favor of dysfunction of BBB has been obtained from post-mortem study and high-resolution MR imaging in life [13].

The present study showed that dysregulation of angiopoietins with a rise in levels of ANG-2 and fall in ANG-1 with altered ratio of ANG-1: ANG-2 occurs in CM as well as in non-cerebral complications. It signifies that endothelial dysfunction plays an important role in both CM and non-cerebral malaria. Malaria with MODS also showed similar finding.

Further, sequestration of pRBC cause occlusion of brain capillaries resulting in reduction of microvascular blood flow that decreases nutrient supply and oxygen to the brain causing and hypoxia that leads to coma. With response to hypoxia angiogenesis is stimulated and it upregulates expression of several genes including release of ANG-2 [6]. Vessels dilate in response to angiogenic factors that disrupts endothelial cell contacts causing vascular permeability and leak of plasma proteins and other blood components causing brain damage.

Different endothelial beds may be responsive differentially

to exogenous stimuli and ANG-1 and ANG-2 dysregulation [6]. Anatomically, the endothelium of brain, kidney and lungs are non-fenestrated continuous endothelium whereas it is non-continuous and fenestrated in liver and bone marrow [6]. Therefore, we did not get significant alteration in patients with jaundice and anemia whereas we got low level of ANG-1 and high level of ANG-2 among the patients of CM and ARDS [14].

The results of the current study also showed that plasma ANG-1, and ANG-2 are biomarkers of SM both among CM and non-cerebral malaria as well as SOD and MODS. ANG-2, ANG-1, and the ratio of ANG-2: 1 was significantly different among UM and SM as well as SM with MOD and SOD. ROC curve analysis indicated good diagnostic accuracy in discriminating UM from SM and between survivors and non-survivors. ANG-2 and ratio of ANG-2 and 1 are found as good predictors of mortality among patients with SM. Hence, it can be used to differentiate the patients of SM with high risk requiring ICU treatment.

The ability of angiopoietins to distinguish between uncomplicated and complicated malaria has been established in four different geographic regions e.g., Africa, Thailand, Malawi, and central India and populations including children [5,15-17]. The present study from Eastern part of India also showed that angiopoietins can discriminate not only survivors and non-survivors but also different types of SM.

Patients of SM require increased organ support management at ICU and face high mortality. Therefore, subsequent assessment of the severity, prognosis and point-of-care indicators of SM remains a challenging task [18]. The present study adds to the value of Angiopoietins by showing that it is helpful in triaging the patients SM. Further, it can be used as a tool at peripheral hospital to refer such patients to higher centers for intensive treatment. However, the cost of the test may be a limitation for its wide use.

In summary, this study showed that dysregulation of angiopoietin occurred in CM either alone or in combination with other complications implicating its possible role in pathogenesis and demonstrated that plasma ANG-1 and 2 levels are reliable biomarkers of SM that can be utilized for prognosis, diagnosis, and disease management.

References

1. World malaria report 2019.
2. World Health Organization. Communicable Diseases Cluster: Severe falciparum malaria. Trans Roy Soc Trop Med Hyg. 2000; 94: S1-S90.
3. Mohapatra MK. The natural history of complicated falciparum malaria- A prospective study. J Assoc Physicians India. 2006; 54: 848-853.
4. Mohapatra MK, Dash LK, Mishra NR, Agrawala RK. Profile of seizures in adult falciparum malaria and the clinical efficacy of phenytoin sodium for control of seizures. Asian Pacific J Trop Dis. 2012; 2: S558-S562.
5. Conroy AL, Phiri H, Hawkes M, Glover S, Mallewa M, Seydal KB, et al. Endothelium based biomarkers are associated with cerebral malaria in Malawian children: a retrospective case-control study. PloS One. 2010; 5: e15291.
6. Fiedler U, Augustin HG. Angiopoietins: a link between angiogenesis and inflammation. Trends Immunol. 2006; 12: 552-558.
7. Rénia L, Howland SW, Claser C, Gruner AC, Suwanarusk R, Teo T, et al. Cerebral malaria mysteries at the blood-brain barrier. Virulence. 2012; 3: 193-201.
8. Mohapatra MK, Das SP. The Malaria Severity Score: a method for severity

- assessment and risk prediction of hospital mortality for falciparum malaria in adults. *J Assoc Physicians India*. 2009; 57: 119-126.
9. ab 99971. Angiopoietin-2 Human Elisa Kit, Abcam Inc. 2012.
 10. ab 99972. Angiopoietin-1 Human Elisa Kit, Abcam Inc. 2012.
 11. World Health Organization. Guidelines for the treatment of Malaria. Third Edition. 2015.
 12. Yuan HT, Khankin EV, Karumanchi SA, Parikh SM. Angiopoietin-2 is a partial agonist/antagonist of Tie-2 signalling in the endothelium. *Mol Cell Biol*. 2009; 29: 2011-2022.
 13. Mohanty S, Benjamin LA, Majhi M, Panda P, Kampodeni S, Sahu PK, et al. Magnetic resonance imaging of cerebral malaria patients reveals distinct pathogenetic process in different parts of brain. *Clin Sci Epidemiol*. 2017; 2: e00193-e00217.
 14. Pham T, Punsawad C, Glaharn S, DeMeyer SF, Viriyavejakul P, Steen PE. Release of endothelial activation markers in lings of patients with malaria associated acute respiratory distress syndrome. *Mal J*. 2019; 18: 395.
 15. Jain V, Luchchi NW, Wilson NO, Blackstock AJ, Nagpal AC, Joel PK, et al. Plasma levels angiopoietin-1 and -2 predict cerebral malaria outcome in Central India. *Mal J*. 2011; 10: 383.
 16. Conroy AL, Lafferty EI, Lovegrove FE, Krudsood S, Tangpukdee N, Liles WC, et al. Whole blood angiopoietin-1 and -2 levels discriminate cerebral and severe (non-cerebral) malaria from uncomplicated malaria. *Mal J*. 2009; 8: 295.
 17. Lovegrove FE, Tangpukdee N, Opoka RO, Lafferty EL, Rajwans N, Hawkes M, et al. Serum angiopoietin-1 and -2 levels discriminate cerebral malaria from uncomplicated malaria and predict clinical outcome in African children. *PLos One*. 2009; 4: e4912.
 18. Mohapatra MK, Thomas AG, Bariha PK, Patel DK. Serum procalcitonin as a triage tool for severe *Plamodium falciparum* malaria. *J Trop Dis*. 2013; 1: 4.