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Original Articles

Albumin Cobalt Binding or Ischaemia Modified Albumin: a Test of Great Prognostic Value in Malaria

Kinjalka Ghosh[1], M.G. Muddeshwar[1], Manoj Lokhande[1] and Kanjaksha Ghosh[2]

[1] Department of Biochemistry, Indira Gandhi Government Medical College (IGGMC), Nagpur, Maharashtra- 440018. India.
[2] Surat Raktadan Kendra and Research Centre, Surat 395002, Gujrat. India.

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Abstract. ***Background:*** **We evaluated albumin cobalt binding (ACB) assay also known as Ischaemia Modified Albumin (IMA) assay as a prognostic marker for severe malaria in a medical college setting.**
Methods: **Consecutive adult patients admitted with both vivax and falciparum malaria were evaluated with ACB assay at the time of admission. Detailed work up and individual patient directed management were instituted in addition to immediate artemisin based antimalarial therapy.**
Results: **100 consecutive patients (50 with vivax and 50 with falciparum malaria) were evaluated. The reference range for ACB assay was established using 50 adult healthy (25 male and 25 female) individuals. 16 out of 50 p. Falciparum-Infected developed complicated malaria. None of the P Vivax patients developed complicated malaria. All malaria infected patients had high ACB levels (P<0.0001). There was a stepwise increase in ACB levels from healthy volunteers to different categories of malaria (P<0.0001) without any overlap.**
Conclusion: **ACB has the potential to be used as a robust simple and inexpensive prognostic marker for organ dysfunction in severe malaria even if an evaluation at multiple sites with a bigger number of patients should be initiated for final recommendation.**

Keywords: Malaria, Complicated malaria, Cobalt albumin binding assay, Vivax Malaria, Falciparum malaria.

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Correspondence to: Kinjalka Ghosh. Assistant Professor. Department of Biochemistry, Seth GS Medical College and KEM Hospital, Parel Mumbai 400012, India. E-mail: kinjalka@gmail.com

Introduction. Malaria especially falciparum malaria is a very common disease in India.[1] If not detected and treated early it may lead to serious complications like cerebral malaria, renal failure, pulmonary oedema and eventually multi-organ failure and death.

Most of the complications of falciparum arise out of its unique property of cytoadherence and rosetting of the infected RBCs to the endothelial cells of capillaries in the microvasculature of various organs, thus sequestering the parasite in those tissues and at the same time avoiding detection and destruction.[2,3]

These bindings eventually lead to the accumulation of parasitized cells in the local postcapillary microvasculature and block the blood flow, limiting the local oxygen supply[3] creating acidosis, cell necrosis and oxidative

damage to the vital tissues due to free radical generation.

Ischemia Modified Albumin (IMA) or albumin cobalt binding assay (ACB) is a marker for oxidative stress and ischemia.[4] It has been approved by the FDA as a marker for ischemia in coronary artery disease. IMA is an albumin which has altered binding capacity to bind transition metals like cobalt (Co), copper (Cu) and nickel (Ni), and similia in its N-terminus region. It is produced during ischemia or oxidative stress when a series of chemical reactions take place altering the N-terminus (N-Asp-Ala-His-Lys) of albumin to form IMA, thus making it unable to bind to those metals.[4,5]

In the current study, we evaluated a cohort of patients affected by both falciparum and vivax malaria, some of them developing various complications during their infection, with an albumin cobalt binding assay to see whether the levels of this analyte correctly identify the complicated malaria patients.

Material and Methods. We collected 5 ml of venous blood from 100 (62 males and 38 females aged between 28 and 61 years) patients with parasitologically confirmed malaria (slide Positive), 50 of which were due to P falciparum infection from the medicine ward on admission. The parasitaemia was also confirmed by immunochromatographic tests, which detected the species of the parasites.

Routine biochemical tests for liver and renal function were done along with complete blood count and peripheral smear in addition to testing for IMA. 5 ml of venous blood was also collected from 50 healthy individuals (30 males and 20 females aged between 22-48 years) who did not have any history of diabetes, coronary artery disease, renal or hepatic disease who acted as controls for measuring the serum Ischemia Modified Albumin levels for normal reference range. Consent from patients and volunteers were obtained, and the study was sanctioned by institutional review board.

Out of 5 ml of venous blood drawn, 1 ml was put in an EDTA bulb for CBC and the rest was collected in a plain bulb, allowed to clot over 15 minutes then centrifuged at 3000g within 1 hr of collection and the serum obtained was tested immediately. Following tests were conducted on all patients and controls:

1. Complete Blood Count (CBC), done on Agappe Hematology Analyzer.

2. Routine biochemical tests for LFT and RFT were done on an automated analyzer (Erba's XL 300,) using standard methods.

3. ACB (IMA) Assay was done colorimetrically as follows:[6] 200 µl of serum was mixed with 50 µl of $CoCl_2$ (1g/L). Mixed vigorously for 10 mins.

50µl of dithiothreitol (DTT) (1.5g/L) was then added. The reaction was allowed to go on for 2mins then it was stopped by adding 0.9N NaCl. Then it was read colorimetrically at 470 nm. The Same procedure was carried out for the blank except for adding DTT.

The concentration of ACB is measured colorimetrically at 470 nm in a standard spectrophotometer and read as ABSU/ml (absorbance units). Standard curves for cobalt levels were made between 6-60 ugm/ml of cobalt chloride where the curve maintains its linearity. The ABSU units also allow one directly to read the amount of free cobalt assessed in the test. The statistical significance was evaluated by student's t-test.

In addition, malaria infection and species identification in all the patients were diagnosed by examining thick and thin blood smear as well as immunochromatography test. (FalciVax, Tulip Diagnostics, Goa, India).

Results. The levels of ACB (IMA) were found to be significantly higher ($p<0.0001$) in all the malaria patients (0.56 ± 0.13) as compared to the healthy controls (0.24 ± 0.04). (**Figure 1**). The levels of ACB correlated very well with the degree of parasitaemia (**Fig 2**, $r=0.97$ $P<0.0001$).

There was also a highly significant ($P<0.0001$) difference in the IMA levels amongst the two types of malaria; falciparum (0.69 ± 0.05) and vivax (0.44 ± 0.06) ABSU (**Figure 2**). The lowest levels of ACB (IMA) amongst falciparum and vivax cases were found to be 0.52 and 0.37. There was also a correlation between at admission ACB levels with the development of complications (**Figure 3**).

ABSU respectively, whereas the maximum IMA level in a normal healthy individual was 0.34 ABSU. So there was no overlap between the malaria cases and normal individuals (**Figure 1**).

Sixteen of the 50 falciparum malaria patients suffered from various complications like ARF (acute renal failure) and cerebral malaria. There

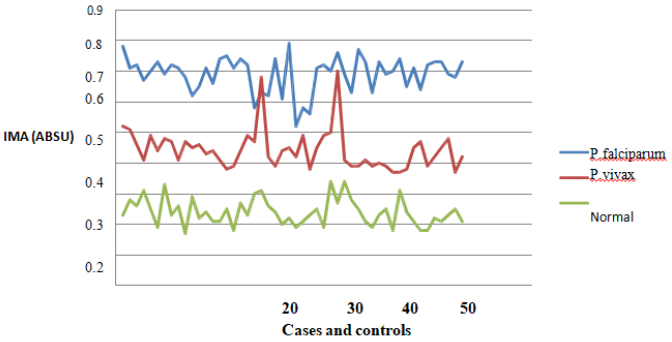

Figure 1. ACB(IMA) Levels in controls and in malaria patients.

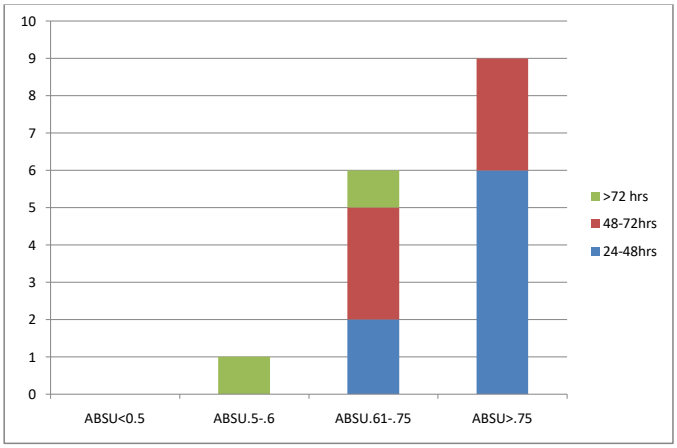

Figure 3. Showing complications in 16 cases (Y axis)of P Falciparum malaria cases correlated with ABSU(X axis) values on different post admission days. (ABSU < 0.5=0, 0.5-0.6=19, 0.61-.75=17, > 0.75 =14).

was one casualty due to cerebral malaria. All of them had extremely high IMA levels as compared to the other cases (lowest IMA – 0.60 ABSU) along with high levels of creatinine and low platelets (**Table 1**). Details of complicated cases are presented in **Table 2**.

Discussion. The present study has shown that there is a significant rise in the levels of IMA in malaria patients, more so for falciparum malaria compared to other healthy people. The levels of IMA were found to have increased nearly 3- fold in cases of complicated malaria (0.74 ABSU) in comparison to the control group (0.24 ABSU). IMA (ACB) Levels in malaria patients have not been studied to a great extent to evaluate its potential as a prognostic marker for heavy parasitaemia and complications. However, IMA was found to be raised in various inflammatory conditions[4] and needs to be assessed for its applicability as a prognostic marker for various inflammatory and autoinflammatory conditions.

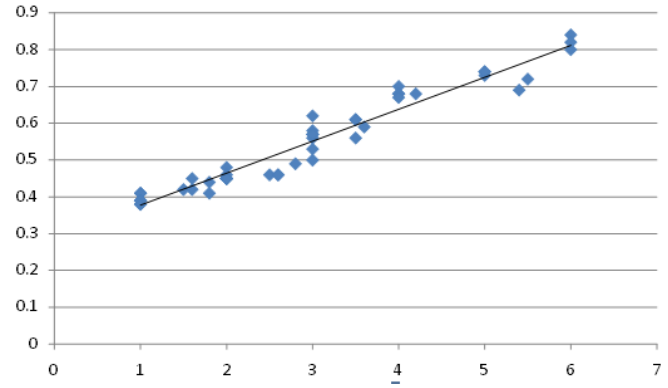

Figure 2. Correlation between P. falciparum parassitaemia in % (X axis) and IMA levels in ABSU/ml (Y axis). Correlation coefficient R^2=.9728, P<0.00001

Today IMA is not a very novel marker anymore; it was found for the first time in 1990 by an emergency doctor who had specially studied a rapid blood test to recognise the myocardial ischemia, later on, it was investigated in great details to serve as an early and reliable marker for myocardial ischemia.[5,7,8,9] It is now one of the United States FDA approved markers for myocardial ischemia.

Ischemia Modified Albumin or Albumin Cobalt Binding Test has come a long way from its discovery in 1990 as an early marker unique to coronary ischemia to being a marker of generalised ischemia and oxidative stress. The main drawback of this test is it lacks specificity.

So if any ischemic process or oxidative damage is going on in our body which would ultimately lead to oxidative stress and tissue damage, IMA is going to be elevated.

So IMA is not going to serve any purpose in diagnosing malaria, we already have many sensitive and specific tests like the various antigen detection tests (Plasmodium LDH, HRP) or PCR for that. However, what IMA can do is tell us when a malaria patient is going to end up in renal failure or cerebral malaria or any other complication arising from an ischemia and oxidative stress that is central to its pathophysiology.

Thus the role of IMA as a marker in malaria is not diagnostic but more of prognostic nature. In the present study we could so a direct correlation between degree of parasitaemia, and complications with ACB levels at presentations. One of the weak points in our study is that we did not serially measure IMA levels in these patients. However, the present study should be considered preliminary though results are encouraging.

Table 1. Patients and their mean+/ 1 Sd IMA levels with mean creatinine and albumin concentration.

Tests/ Samples (n)	**IMA** (ABSU/ml)	**Creatinine** (mg/dl) dl)	**Albumin** (gm/dl)	**Platelets** (cells/µl)
Normal (50)	0.24+/-0.04	0.56	4.8	2,28,000
Malaria (100)	0.56+/-0.13	1.3	3.8	1,08500
Falciparum (50)	069+/- 0.05	1.8	3.7	76,290
Vivax (50)	0.44+/-0.06	0.96	4.5	1,64,800
Complicated Malaria (16)	0.74+/-0.08	2.8	3.2	55,000

Table 2. Different presentations of complicated malaria.

Number of cases	Number	IMA (ABSU)
Complicated Malaria	16	0.74+/-0.08
Cerebral Malaria	10	0.78+/-0.06
Multiorgan failure	6	0.80+/- 0.09
Renal failure only	4	0.67+/- 0.065
hepatitis	4	0.70+/- 0.1
Death	6	

Total number of complicated malaria is 16, as they have a combination of problems and some of them expired hence the grand total is more than 16.

Being able to predict which of the malaria patients is going to land up in complications is an important issue if we know it from high levels of ACB in these patients that they are likely to develop complications as the present study showed then adequate measures could be taken to prevent it. This procedure would go a long way in reducing the number of deaths due to malaria in an endemic region like that of ours. The test is simple, inexpensive and rapid. This test can be adopted in any laboratory in a developing country as well as in developed one.

More costly ELISA variation of this test measuring the altered albumin antigen is available (Sun Red Biotechnology, Shanghai, China). Recently sensitivity of the test has also improved, and the test has been developed in an ELISA plate format by optimising DTT (Dithiothreitol) amount in a phosphate buffered (ph 7.4) environment.[10]

However, whether this improved version of the albumin cobalt binding (ACB) test, which the authors preferred to call cobalt-albumin binding test (CAB test), needs to be evaluated for various conditions before it can be recommended. ACB is an inexpensive test in India one ACB test costs no more than 5 p (UK) per test.

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