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Original Research Article

ASSESSMENT OF ISCHEMIA MODIFIED ALBUMIN AS A BIOMARKER OF OXIDATIVE STRESS IN SUSPECTED SUBCLINICAL ISCHEMIC EPISODES IN HYPERTENSIVE BLACK ZAMBIANS AT UNIVERSITY TEACHING HOSPITAL, LUSAKA.

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Abstract: Background: The detection of ischemia prior to infarction is a challenging concept. Studies have shown ischemia modified albumin (IMA) to be a sensitive marker of ischemia, and it has been suggested that IMA could be an early marker to help detect ischemic stroke and ruling out patients with acute coronary syndrome. The free radicals generated in the Fenton reaction cause damage to the N terminal of albumin, this damage causes a reduction in the binding affinity of albumin for transitional metals (e.g. cobalt). The reduced binding affinity of albumin for transitional metals is the principle of some measurement methods for ischemia modified albumin (IMA). We set out to determine whether IMA could be used as a biomarker of oxidative stress in suspected sub-clinical ischemic episodes in hypertensive black Zambian. **Materials and method:** A total of 63 study participants were enrolled, 42 were hypertensive (21 without stroke and 21 with stroke), and 21 were normotensive age matched controls. Ischemia modified albumin levels were measured in all the study participants using IMA ELISA assay. Statistical analysis was done using SPSS (version 23) to compare the mean difference in IMA levels between the participants. ANOVA test and Student's t test were used to detect any significant differences in mean IMA levels among the three study groups. **Results:** Participants with hypertension and stroke had a mean IMA level of 10.721 ng/mL, those with hypertension only had 10.15 ng/mL while the normotensive controls had 6.723 ng/mL. ANOVA showed a significant difference in mean serum IMA between the three groups (F=85.259). The student t test showed a significant difference between hypertensives and the controls (t=12.833, p<0.0002)) but not between the hypertensives with stroke and without stroke (t=1.679). **Conclusion:** Mean IMA levels were higher in hypertensives and hypertensives with stroke than in normotensives. However, IMA levels in hypertensives with stroke and hypertensives only were not statistically different; suggesting that IMA levels could not be used to predict which hypertensive patient was more at risk of developing stroke. Further, stroke in our black Zambians may be caused more by hemorrahagic rather than ischemic episodes

Key words: Ischaemia modified albumin (IMA), Oxidative stress, Free radical, Transitional metal, Fenton reaction

Introduction: The detection of ischemia prior to infarction is a challenging concept, however Ischemia modified albumin (IMA) testing is promising to be a major breakthrough[1]. Studies have shown that IMA was a sensitive marker of ischemia, and it has been suggested that IMA could be used as an early marker to help detect stroke, especially the ischemic type[2] and in ruling out patients with acute coronary syndrome[3].

The precise mechanism of IMA production is yet unknown[4]. Bar-Or D hypothesized that; during periods of ischemia the body gets into an acidic state and the proteins that migrate along the ischemic area as the blood flows release divalent copper ions (Cu^{2+}) , and the Cu^{2+} are scavenged by albumin through tight binding to the N-terminus. The released Cu^{2+} are reduced to monovalent copper ions (Cu^{+}) in the presence of reducing substances such as ascorbic acid, and the Cu^{+} produced by this mechanism react with oxygen to generate superoxide free radicals, which are converted to hydrogen peroxide (H_2O_2) by superoxide dismutase. Under normal conditions in vivo, hydrogen peroxide is converted to water and oxygen by catalase. However, in the presence of Cu^{+}, H_2O_2 is converted to hydroxyl free radicals, through the Fenton reaction[5]. These free radicals alter the N-terminus of albumin which decreases its binding capacity for transitional metals, this altered albumin is referred to as Ischaemia Modified Albumin (IMA)[6]. This reduced binding of transitional metals causes release of more Cu^{2+}, thus generates more free radicals through the Fenton reaction. The resulting vicious cycle induces a sudden increase in IMA[7]. The free radicals oxidize membrane proteins on the inner part of the blood vessels thereby inducing atherosclerosis[8].

Oxidative stress has been identified as an important factor associated with hypertension[9]. The imbalance caused due to increased reactive oxygen species (ROS) production and/or reduced antioxidant systems results in oxidative stress[10]. This induced sclerosis contributed to increased peripheral vascular resistance, a hallmark of hypertension[11]. The increased peripheral vascular resistance may also have resulted from enhanced vasoconstriction and/or impaired endothelium dependent vasodilatation[12]. The impaired vasodilation seen during hypertension has been attributed primarily to endothelial dysfunction[13]. Endothelial dysfunction is a consequence of impairment in nitric oxide (NO) synthesis and/or bioavailability. Oxidative stress has been hypothesized to play a role in modulating endothelial dysfunction[7] by generating ROS that scavenge NO by forming highly reactive peroxynitrite radicals[14] and also by uncoupling endothelial nitric oxide synthase (eNOS) resulting in impaired endothelium dependant relaxations[15]. Worldwide hypertension is estimated to cause 7.5 million deaths, about 12.8% of total deaths and is implicated in over 50% of ischemic strokes[16]. The morbidity and mortality remains high despite formulation of more potent drugs for blood pressure control, the reason for this may be that most of the currently available techniques to investigate hypertension related complications are only able to detect a complication after cell necrosis has occurred e.g. use of cardiac enzymes in diagnosis of myocardial infarction[1].

An argument is made herein that in hypertensives the degree of ischemia modified albumin elevation denotes the degree of endothelial dysfunction and oxidative stress which in turn signify the severity of hypertension which directly relates to the occurrence of complications. This research attempted to establish the role of ischemia modified albumin in the early detection of

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subclinical ischemic processes that may occur in hypertensive persons because of the ongoing oxidative stress. These subclinical ischemic episodes if left unchecked culminate to overt clinical complications such as stroke. Limited data is available on IMA testing and its application in the Zambian context. This study was undertaken in an attempt to address this gap in research, contribute to the body of knowledge in this area and more importantly to generate data that might aid in the development of more cost effective approaches in management of hypertension and its complications, thus leading to reduction in associated morbidity and mortality.

Materials and Methods: This study was conducted in the adult medical emergency unit of the department of internal medicine, University Teaching Hospital, Zambia; after being approved by the University of Zambia Bio-medical Research Ethics Committee (UNZABREC), and informed consent was obtained from all participants. We studied 42 participants recruited from the medical emergency unit with an age range 25 - 70 years with hypertension, 21 of these had hypertension only while another 21 has hypertension with stroke. In addition, 21 apparently healthy individuals matched for age were included as controls. The exclusion criteria included pregnancy, cancer, infections, end stage renal disease, liver disease and uncontrolled diabetes and history of recent surgery. All subjects were subjected to:

1. A relevant history: which focused on establishing any history of recent surgery, when they were diagnosed with hypertension, presence of chronic diseases like cancer, rheumatologic conditions, establish likely hood of pregnancy or presence of infection, liver, renal pathologies or diabetes and their drug history.
2. Clinical examination for signs especially for signs of cerebro vascular stroke (paresis, paralysis, loss of sensation, abnormal speech).
3. Blood samples each approximately 4mls were collected by venepuncture in the cubital fossa using a 21G bore needle and 5ml syringe after candidate met the inclusion criteria. The collected blood was then transferred into a 4ml blood volume lithium heparin anticoagulated vacutainers that was numbered with the unique participants' assigned serial number as recorded on the questionnaire. The blood specimen was then transported to the UTH's clinical chemistry laboratory in under 30minutes of collection for processing. At the lab the samples were processed for: routine laboratory tests (liver function tests, renal function). The remaining blood specimens were coagulated for 2 hours at room temperature and then centrifuged at 1000 x g revolutions for 20 minutes. The serum was then aliqouted and then stored in a freezer at -20^{o}C until the specimens was required for IMA analysis. IMA was measured using the IMA ELISA kit, abx250303. The assay is based on the fact that ischemia modified albuimn has reduced binding for transitional metals such as cobalt. The IMA ELISA kit used applied the quantitative sandwich enzyme immunoassay technique. The microtiter plate had been pre-coated with a monoclonal antibody specific for IMA. Standards or samples were then added to the microtiter plate wells and IMA was present, it bound to the antibody pre-coated wells. In order to quantitatively determine the amount of IMA present in the sample, a standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody, specific for IMA was added to each well to "sandwich" the IMA immobilized on the plate. The microtiter plate under went incubation, and then the wells were thoroughly washed to remove all unbound components. Next, substrate solutions were added to each well. The enzyme (HRP) and substrate were allowed to react over a short incubation period. Only those wells that contain IMA and enzyme-

conjugated antibody exhibited a change in color. The enzyme-substrate reaction was terminated by addition of a sulphuric acid solution and the color change was measured spectrophotometrically at a wavelength of 450 nm. A standard curve was plotted relating the intensity of the color (O.D.) to the concentration of standards. The IMA concentration in each sample is interpolated from this standard curve.

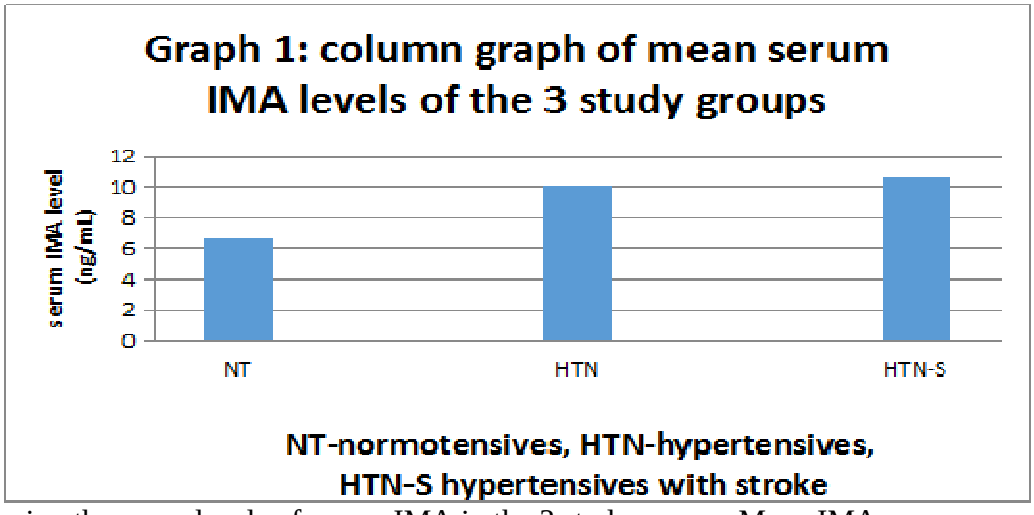
Statistical analysis: The clinical and laboratory results obtained were statistically analyzed using SPSS (version 23) for windows. ANOVA and Student t tests were performed to detect any differences in mean IMA levels among the study participants. All statistical tests were performed at 95% confidence interval with p-value of <0.05 to determine statistical significance.

Results: ANOVA showed a significant difference in mean serum IMA levels between the three groups (F value 85.259) (table 2). As revealed by student t test, difference among controls and hypertensive's without stroke was significant (t value 12.833), and p < 0.0002 but there was no difference between hypertensives without stroke and hypertensive's with stroke (t value 1.679) (table 3). The IMA assay had a diagnostic accuracy 86%, a negative predictive value of 71%, a positive predictive value of 97% and sensitivity of 81% and specificity of 95% when used to detect presence of oxidative stress. (table 4). IMA had a diagnostic accuracy of 55%, a negative predictive value of 63%, a positive predictive value of 53% and sensitivity of 86% and specificity of 24% when used to predict occurrence of stroke in hypertensive patients. (Table 5).

Table 1: Mean serum IMA levels of the three study groups

Group	Sample size	Mean serum IMA (ng/mL)	Std deviation	P value when compared to normotensive
Normotensive	21	6.723	0.733	
Hypertensive only	21	10.15	0.98	< 0.0002
Hypertensive with stroke	21	10.721	1.211	< 0.000

Table shows a high serum IMA in hypertensives and hypertensive's with stroke compared to normotensives. This correlates with the increasing degree of oxidative stress across the groups.



Graph showing the mean levels of serum IMA in the 3 study groups. Mean IMA. Levels were at 6.723 in normotensives, 10.15 in hypertensives only and 10.721.

Table 2: ANOVA parameters

Source	Df	SS	MS	F
Between	2	196.371	98.186	85.259
Within	60	61.939	1.032	
Total	62	258.31		

At α level 0.05 and degree of freedom 2 and 60, F table critical value = 3.15. Since our calculated F value (85.259) is greater than the table critical value (3.15), we reject the null hypothesis that there was no difference in mean serum IMA levels between the 3 groups and accept the alternative hypothesis that there was a significant difference in the serum IMA levels between the 3 groups. This was due to the raised degree of oxidative stress between normotensives, hypertensives and hypertensives with stroke.

Table 3: Student t test results for IMA differences between 2 groups

Group	t-value
IMA level of normotensives and hypertensive's	12.833 (P < 0.0002)
IMA level of hypertensives and hypertensives with stroke	1.679

At α level 0.05 and degree of freedom 40, t table critical value = 1.684. The t test results revealed that the mean serum IMA level difference among the normotensive group and hypertensive group was significant (p < 0.0002), with the hypertensive group having higher IMA levels than the normotensive group, but the difference between hypertensive group and the hypertensive group with stroke was not statistically significant at α level 0.05.

Table 4: IMA test performance for predicting oxidative stress in hypertensives at serum IMA cut off level of 9 ng/mL

Clinical condition status	hypertensives	normotensives	Total tests
IMA test positive	34	1	35
IMA test negative	8	20	28
Total	42	21	63

From the table above IMA assay diagnostic sensitivity was 81%, diagnostic specificity 95%, positive predictive value 97% and negative predictive value71% and had a diagnostic accuracy of 86%.

Table 5: IMA test performance for predicting likelihood stroke in hypertensives at serum IMA cut off point of 9 ng/mL

Clinical condition status	Stroke present	Stroke absent	Total tests
IMA test positive	18	16	34
IMA test negative	3	5	8
Total	21	21	42

From the table above IMA assay sensitivity is 86%, specificity is 24%, positive predictive value is 53% and negative predictive value is 63% and a test diagnostic accuracy of 55%.

Discussion: Increased oxidative stress is one of the principal mechanisms by which hypertension exert its pathological influence leading to complications such as stroke[11]. IMA assay is a sensitive marker for early detection of stroke and levels tend to be higher in ischemic stroke than in hemorrhagic stroke[17]. IMA is still a relatively new biomarker on the market with a great deal of information yet to be known about it, thus it's imperative that a guarded approach is used when dealing with it. The currently accepted strength of IMA lies in its negative predictive value for excluding the presence of ischemia[18]. Our study showed IMA to have a negative predictive value of 71% and diagnostic accuracy of 86% at an IMA cut off value of 9

ng/mL. These figures are reasonably high and may be IMA could be considered for use as a biomarker in the evaluation of ischemia before the onset of infarction in hypertensive patients especially if there are no overt clinical features.

Our results showed an increased IMA levels in hypertensives (10.15 ng/mL) and hypertensives with stroke (10.721 ng/mL) compared to the normotensive (6.723 ng/mL) study group. This was confirmed by our statistical computation of ANOVA F value of 85.259 (which is greater than the critical F table value of 3.15 at 0.05 confidence interval and degree of freedom of 2 and 60) and the t test value of 12.833 (which is greater than the critical t test table value of 1.684 at 0.05 confidence interval and degree of freedom of 40) and $p<0.0002$. The increased IMA level in the hypertensive groups was most likely due to increased oxidative stress in hypertensives whether with stroke or not[17]. Oxidative stress induces sclerosis which contributes to increase in peripheral vascular resistance and is thus an important factor in the pathogenesis of hypertension. Oxidative stress generates reactive oxygen species (ROS) associated with ischemia reperfusion injury, these ROS are the chief factors involved in modifying the metal binding domain of albumin molecule at its N-terminal residues thus reducing its affinity to bind transitional metals e.g. cobalt[5]. This reduced binding of transitional metals causes release of more Cu^{2+}, thus generates more free radicals through the Fenton reaction. The resulting vicious cycle induces a sudden increase in IMA[7]. The free radicals oxidizes membrane proteins on the inner part of the blood vessels thereby inducing atherosclerosis which increases vascular resistance, a major contributor to development of hypertension[8].

Our study showed that IMA levels were not statistically different between the hypertensive's with stroke (10.721 ng/mL) and the hypertensive's without stroke (10.15 ng/mL) as deduced from the statistical computation of the t test value, between the 2 groups, of 1.679 (critical t test value of 1.684 at 0.05 confidence level and 40 degrees of freedom). Thus IMA could not distinguish between stroke and hypertension. This result is in agreement with our literature review that IMA is a non specific indicator of oxidative stress, but we could also infer from this result that probably the correlation between hypertension, oxidative stress and the occurrence of stroke is non-linear. Other factors that could explain our observations were found in the research by Gunduz et al in 2011[19]. According to Gunduz et al, despite IMA being a sensitive marker for ischemia, its sensitivity decreases rapidly over 72 hours, the sensitivity decreases even more rapidly in conditions associated with transient and reversible ischemia. Another factor responsible for the possible false negative IMA value is the presence of lactic acid in stroke patients secondary to prolonged ischemia and acidosis which may be both metabolic and respiratory. Elevated lactic acid levels have been shown to be associated with a decrease in IMA levels, the cause of which is not known. A possible third cause maybe delayed presentation to the emergency room by which time IMA would have started to disintegrate.

Mentese et al in 2008[20], showed that IMA levels are raised in a number of acute ischemic conditions such as cerebral infarction, myocardial infarction and pulmonary and mesenteric infarction, suggesting that IMA may be useful as a diagnostic marker of ischemia. The currently accepted strength of IMA lies in its negative predictive value for excluding presence of ischemia[18]. Our study showed that IMA increases provided some degree of oxidative stress is present whether or not infarction occurs. This is inferred from the IMA results of hypertensive's and those with hypertension and stroke that showed similar results with no statistical difference, t value 1.679 (at 40 degrees of freedom and α level 0.05, t table value = 1.684). This was in agreement with our literature review that IMA is a non specific biomarker of oxidative stress.

The results may also suggest that the stroke may be associated with hemorrhagic episodes rather than ischemic episodes in our Zambian patients, and the lesions may be stabilized with alternative blood supply to the ischemic area. Thus, it would be ill advised to use IMA as a standalone test to predict occurrence of stroke in hypertensive patients because its diagnostic accuracy when used for this purpose was 55%, with a negative predictive value of 63%, a positive predictive value of 53% with sensitivity and specificity at 86% and 24% respectively. Most of these figures that are below the 70% mark, making IMA not a very suitable diagnostic test for this purpose. However IMA may be considered for use in the emergency room in conjunction with CT brain for the diagnostic assessment of suspected stroke, to exclude ischemic stroke in patients with low clinical probability. In this context, we would need to use an IMA cut off point value that is low so as to increase its sensitivity at the expense of specificity. This is clinically justifiable if we consider the implications of over inclusion of patients because of the raised sensitivity against the morbidity, mortality and expense of treatment, of a patient who was not prioritized and developed stroke because the IMA test with a balanced sensitivity and specificity triaged the patient into moderate to low risk category.

Conclusion: We demonstrated raised serum mean IMA levels in hypertensives (both with and without stroke) compared to normotensives signifying increased oxidative stress in the hypertensive participants. However statistically similar serum mean IMA values in the hypertensives with stroke and hypertensives without stroke suggested that IMA could not be used to predict the risk of stroke development in hypertensive patients. It could also suggest that hemorrhagic episodes were the most common cause of stroke in Black Zambians.

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References

1. Sinha MK, Roy D, Gaze DC, Collinson PO and Kaski, JC, Role of “Ischemia modified Albumin”, a new biochemical marker of Myocardial Ischemia, in the early diagnosis of acute coronary syndrome. Emerg. Med. J., 2004, 21: 29-34.
2. Mohamed SG, Nermeen HZ, Nany Hassan AE, Evaluation of the role of ischemia modified albumin (IMA) as a new biochemical marker for differentiation between ischemic and hemorrhagic stroke. Alexandria Journal of Medicine., 2015, 51: 213 - 217.
3. Christenson RH, Duh SH, Sanhai WR, et al, Characteristics of an albumin cobalt binding test for assessment of acute coronary syndrome patients: a multicenter study. Clin Chem., 2001, 47: 464-470.
4. Collinson PO, Gaze DC, Ischemia-modified albumin: clinical utility and pitfalls in measurement. J Clin Pathol., 2008, 61:1025-8.
5. Bar-Or D, Winkler JV, Van Benthuysen K, Harris L, Lau E and Hetzel FW, Reduced albumin cobalt binding with transient myocardial ischemia after elective percutaneous transluminal coronary angioplasty : A preliminary comparison to creatine Kinase-MB, myoglobin and Troponin I. Am. Heart J., 2001, 141: 985-991.
6. Laussac JP and Sarkar B, Characterization of the copper (II) and nickel (II) – transport site of human serum albumin.Studies of copper (II) and nickel (II) binding to peptide 1- 24 of human serum albumin by 13C and 1H NMR spectroscopy. Biochemistry., 1984, 23: 2832-2838.
7. Roy D, Quiles J, Kaski JC, Baxter GF, Role of reactive oxygen species on the formation of the novel diagnostic marker ischaemia modified albumin. Heart., 2006, 92: 113-114.

8. Ahn JH, Choi SC, Lee WG, Jung YS, The usefulness of albumin adjusted ischemia modified albumin index as early detecting marker for ischemic stroke. Neurol Sci., 2011, 32: 133 - 8.
9. Ceriello A, Esposito K, Piconi L. Glucose "peak" and Glucose "spike": impact on endothelial function and oxidative stress. Diabetes Res Clinic Pract., 2008, 82(2): 262 - 7.
10. Lee Yoel Moo, Griendling K Kathy, Redox Signaling, Vascular function and Hypertension. Libertpub., 2008: 10(6): 1045 - 1059.
11. Cachofeiro V, Miana M, Heras ND, Fernandez BM, Ballesteros S, Balfagon G, et al, Inflammation: a link between hypertension andatherosclerosis. Curr Hypertens Rev., 2009, 5: 40-48.
12. Puddu P, Puddu GM, Zaca F, Muscari A, Endothelial dysfunction in hypertension. Acta cardiologica., 2000, 55: 221-232.
13. Moraweitz H, Taknow R, Szibor M, Regulation of the endothelial system in the human endothelial cells. J Physiol (Lond). 2001, 525: 761 - 770.
14. Escobales N and Crespo MJ, Early Pathophysiological Alterations in Experimental Cardiomyopathy. PRHSJ., 2008, 27: 307-314.
15. Landmesser U, Price SR, McCann L, Fukai T, Holland SM, Mitch WE, Harrison DG, Oxidation of tetrahydrobiopterine leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. J Clin Invest., 2003, 111(8): 1201 - 9.
16. WHO, WHO Global Health Observatory (GHO) data, available at http://www.who.int/gho/ncd/risk_factors/blood_pressure_prevalence_text/en/ (accessed on 22nd July, 2016).
17. Sameer Abboud H, Labreuche J, Meseguer E, et al, Ischemia modified albumin in acute stroke. Cerebrovasc Dis., 2008,23(2–3):216–20.
18. Aslan D, Apple FS, Ischemia modified albumin: clinical and analytical update. Lab Med., 2004, 35: 1-5.
19. Gunduz A, Turdi S, Menetese A, Karahan SC, et al, Ischemia modified albumin levels in cerebrovascular accidents. Am J EmergMed, 2011, 26(11):874–8.
20. Mentese A, Turdi S, Tophas M, et al, Effect of deep vein thrombosis on ischemia modified albumin levels. Emerg Med J., 2008, 25(12):811–4.