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

Simoonga Peter

A Dissertation submitted to the University of Zambia

In Partial Fulfillment of the Requirement for the award of Masters of Science Degree in Pathology (Chemical Pathology)

THE UNIVERSITY OF ZAMBIA
LUSAKA
(2017)
DEDICATION

To my wife Bertha, my little daughter Natalia Luyando and the entire extended family, for the love and understanding as this period must have been arduous, with the long hours spent at the University Teaching Hospital Chemistry laboratory, on the laptop gathering information from journals and hours spent typing this research.
DECLARATION

This dissertation is the original work of Peter Simoonga.

It has been produced in accordance with the guidelines of the Masters of Science in Pathology (Chemical Pathology) dissertation for the University of Zambia. It has not been submitted either wholly or in part for any other Degree at this or any other University nor is it being currently submitted for any other Degree.

Signed: .................................................................
Peter Simoonga

Date: ........................................................................

Signed: .................................................................
Professor Trevor Kaile (supervisor)

Date: ........................................................................
APPROVAL CERTIFICATION

The University of Zambia approves this dissertation of Peter Simoonga as fulfilling part of the requirements for the award of the Masters of Science Degree in Pathology (Chemical Pathology).

EXAMINERS

Examiner 1: ..................................Signature...............date............... 

Examiner 2: ..................................Signature...............date............... 

Examiner 3: ..................................Signature...............date............... 

Head of Department 
Department of Pathology and Microbiology

Signature: .................................................................

Date: .................................................................
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ABSTRACT

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 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 Zambians.

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. IMA 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.

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).

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.

Key words: Ischaemia Modified Albumin (IMA), Oxidative stress, Free radical, Transitional metal, Fenton reaction.
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<tbody>
<tr>
<td>ACE</td>
<td>Angiotensin Converting Enzyme</td>
</tr>
<tr>
<td>AMEU</td>
<td>Adult Medical Emergency Unit</td>
</tr>
<tr>
<td>Ang II</td>
<td>Angiotensin II</td>
</tr>
<tr>
<td>AT1</td>
<td>Angiotensin Type 1</td>
</tr>
<tr>
<td>AT2</td>
<td>Angiotensin Type 2</td>
</tr>
<tr>
<td>BH4</td>
<td>Tetrahydrobiopterin</td>
</tr>
<tr>
<td>CKMB</td>
<td>Creatinine Kinase Myocardial Band</td>
</tr>
<tr>
<td>DAG</td>
<td>Diacylglycerol</td>
</tr>
<tr>
<td>DALYS</td>
<td>Disability Adjusted Life Years</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial Nitric Oxide Synthase</td>
</tr>
<tr>
<td>H2O2</td>
<td>Hydrogen Peroxide</td>
</tr>
<tr>
<td>IMA</td>
<td>Ischemia Modified Albumin</td>
</tr>
<tr>
<td>IP3</td>
<td>1,4,5-Inositol Triphosphate</td>
</tr>
<tr>
<td>KHB</td>
<td>Rebs-Henseleit Buffer</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate Dehydrogenase</td>
</tr>
<tr>
<td>MAP</td>
<td>Mean Arterial Pressure</td>
</tr>
<tr>
<td>MAPK</td>
<td>Mitogen Activated Protein Kinase</td>
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<tr>
<td>MDA</td>
<td>Malondialdehyde</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger Rna</td>
</tr>
<tr>
<td>NFkB</td>
<td>Nuclear Factor Kappa B</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
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<tr>
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<td>Nitric Oxide Synthase</td>
</tr>
<tr>
<td>OS</td>
<td>Oxidative Stress</td>
</tr>
<tr>
<td>RAAS</td>
<td>Renin Angiotensin Aldosterone System</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SOD</td>
<td>Superoxide Dismutase</td>
</tr>
<tr>
<td>UTH</td>
<td>University Teaching Hospital</td>
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</tbody>
</table>
CHAPTER ONE

INTRODUCTION

1.1 Background

Detection of ischemia prior to infarction is a challenging concept, recent studies have shown Ischemia Modified Albumin (IMA) to be a sensitive marker of ischemia that may help in early detection of ischemic stroke (Mohamed et al, 2014) and in ruling out patients with acute coronary syndrome (Christenson et al, 2007). However, the precise mechanism of IMA production is yet unknown (Gaze et al, 2006). According to Bar-Or D when the body gets into an acidic state the proteins that migrate along the ischemic area in the blood flow release divalent copper ions (Cu$^{2+}$), which are scavenged by albumin through tight binding to the N-terminus. The Cu$^{2+}$ ions are reduced to monovalent copper ions (Cu$^{+}$) in the presence of reducing substances such as ascorbic acid. The monovalent copper ions produced by this mechanism react with oxygen to generate superoxide free radicals, which are converted to hydrogen peroxide (H$_2$O$_2$) by superoxide dismutase. Under normal conditions in vivo, hydrogen peroxide is converted to water and oxygen by catalase. However, in the presence of Cu$^{+}$ ions, H$_2$O$_2$ is converted to hydroxyl free radicals, through the Fenton reaction (Bar-Or D et al, 2000). During periods of ischemia these free radicals alter the N-terminus of albumin which decreases its binding capacity for transitional metals. It is this altered albumin that is referred to as IMA. Albumins’ reduced binding for transitional metals causes the release of more divalent copper ions, thus generating more free radicals through the Fenton reaction. (Laussac et al; 1984). The resulting vicious cycle induces a sudden increase in IMA (Roy et al; 2006). The free radicals oxidize membrane proteins on the inner part of the blood vessels thereby inducing atherosclerosis. Atherosclerosis could be a risk factor for ischemic stroke.(Ahn et al; 2011).

Hypertension is a major risk factor for several cerebrovascular and cardiovascular diseases such as stroke, atherosclerosis, heart failure and end organ damage and it affects at least 1 in 3 adults. (Diaz et al; 2014). According to the 2015 World Heart Federation report, hypertension causes over 50% of ischemic strokes and also increases the risk of hemorrhagic stroke. Oxidative stress has been identified as an important factor associated with hypertension (Ceriello et al; 2008). It
results from an imbalance of increased generation of reactive oxygen species (ROS) and/or reduced function of the antioxidant system (Nickenig and Harrison; 2002). ROS damage the endothelium and thus induces sclerosis which contributes to an increase in peripheral vascular resistance (Cachofeiror et al; 2009). Increase in peripheral vascular resistance is a hallmark of hypertension and may result from impaired endothelium dependent vasodilatation and enhanced vasoconstriction mediated by increased response to vasoconstrictors such as angiotensin II (Ang II) and catecholamines (Puddu et al; 2000). Ang II via AT1 receptors also induces vascular remodeling predominantly in large conduit and small resistance vessels with resultant increase in vascular tone. (Mehta et al; 2007)

Impaired endothelium dependent vasodilatation has been primarily attributed to endothelial dysfunction in the form of impaired nitric oxide (NO) synthesis and/or bio-availability. (Munzel et al; 2005). Oxidative stress contributes to endothelial dysfunction by generating ROS, particularly superoxides that scavenge NO by forming highly reactive peroxynitrite radicals (Roy et al; 2006) and also uncoupling of endothelial nitric oxide synthase (eNOS) thus resulting in impaired endothelium dependent relaxation. (Landmesser et al; 2003). The uncoupled eNOS generates reactive oxygen species instead of NO thereby reducing NO production and further increasing oxidative stress. (Munzel et al; 2005)

The research aimed to establish the role of IMA as an early marker of subclinical ischemic processes that occur in hypertensives secondary to the ongoing oxidative stress. We hoped the results obtained from the study would aid in the development of more efficient and effective hypertension management strategies so as to reduce complications like stroke and myocardial infarction and their associated morbidity and mortality.

1.2 Statement of the problem

Worldwide hypertension is estimated to cause 7.5 million deaths, about 12.8% of total deaths. This accounts for 57 million disability adjusted life years (DALYS) (WHO, GHO; 2015). Hypertension is the single most important risk factor for cardiac related ischemic complications such as stroke, myocardial infarction, nephropathy and peripheral vascular disease according to the World Heart Federation 2015 publication.
Despite its wide prevalence, the pathomechanism’s of hypertension are not well understood. (Munzel et al., 2005). Currently available techniques for investigating and diagnosing hypertension related complications only detect a complication after cell necrosis has occurred e.g. use of cardiac enzymes in diagnosis of myocardial infarction (Turedi et al. 2007). This limited knowledge significantly stymies the development of novel therapeutic interventions and prevention of fatal outcomes, hence the high rates of morbidity and mortality associated with complicated hypertension.

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 has been shown to be directly related to the occurrence of complications. Thus, it may be hypothesized that hypertensive patients may have increased serum ischemia modified albumin levels and the degree to which it increase is directly related to the occurrence of complications.
OBJECTIVES

1.3 Research questions

1. Is IMA elevated in hypertensive black Zambians?
2. Could IMA be utilized as a biomarker of oxidative stress to detect subclinical ischemic episodes in hypertensive black Zambians?

1.4 General objective

To assess IMA levels and determine whether IMA could be used as a biomarker of oxidative stress in suspected subclinical ischemic episodes in hypertension.

1.5 Specific objectives

1. To determine the mean difference in plasma IMA concentration in hypertensive patients (with and without stroke) and normotensive Zambians.
2. To establish whether IMA could be utilized as a biomarker of oxidative stress in suspected subclinical ischemic episodes, using stroke as a proxy of oxidative stress.
CHAPTER TWO

LITERATURE REVIEW

2.1. Ischaemia Modified Albumin (IMA)

Generation of IMA

Albumin is the most abundant circulating protein in human blood with a versatile ability to bind small molecule ligands. (Gaze et al; 2006). It can act as depot and carrier for many endogenous compounds and can render potential toxins harmless. (Bar-Or D et al 2000). Albumin is widely used clinically as a diagnostic or monitoring biomarker in the treatment of diseases and its modified form has also been found to be diagnostically effective in the treatment of acute ischemic stroke (AIS) patients. (Ahn et al; 2011). Modified Albumin referred to as Ischaemic Modified albumin (IMA) has been shown to be a sensitive marker of ischemia, and it could be adopted as an early marker to help detect acute episodes of ischemic stroke (Mohamed et al, 2014). However, the precise mechanism of IMA production is yet unknown (Collinson et al, 2008). Bar-Or D hypothesized that, during periods of ischemia the body got into an acidic state and the proteins that migrate into the ischemic area as the blood flows release divalent copper ions (Cu^{2+}), the Cu^{2+} are then 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_{2}O_{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_{2}O_{2} is converted to hydroxyl free radicals, through the Fenton reaction (Bar-Or D et al, 2001). These free radicals alter the N-terminus of albumin which decreases its binding capacity for transitional metals, this altered albumin is referred to as IMA. (Laussac et al; 1984). This reduced binding of transitional metals causes release of more Cu^{2+}, thus generating more free radicals through the Fenton reaction. The resulting vicious cycle induces a sudden increase in IMA. (Roy et al; 2006). The free radicals oxidize membrane proteins on the inner part of the blood vessels thereby inducing atherosclerosis. (Ahn et al; 2011). The amount of unbound transitional metal e.g. cobalt, is
directly related to an increase in IMA, this unbound cobalt can form a complex with a chromogen (dithiothreitol) to produce a colored product on the test platform which can be measured photometrically. This is the basis of the albumin cobalt-binding (ABC) test. (Christenson et al; 2001). This assay is reported to be positive within 6 to 10 minutes of ischemia, allowing for the detection of ischemia before necrosis and remains so up to 6 hours later, these changes of albumin may not be irreversible. (Sbarouni et al; 2008). The rapid return to normal within a few hours (12 to 24 hours) may be a function of restored perfusion which may facilitate removal of the free radicals. (Talwalker et al; 2008)

Clinical Applications of IMA

I) IMA as a marker of ischemia:

The detection of ischemia prior to infarction is a challenging concept. (Turedi et al.2007). Several studies have showed IMA to be a sensitive marker of ischemia prior to infarction (Mohamed et al; 2014) and that it correlates well with currently used objective markers of ischemia such as lactate levels and isoprostane. (Talwalkar et al; 2008). AIS is one of the leading causes of death and disability worldwide and oxidative stress has been implicated as the fundamental mechanism of brain damage in AIS. (Sims et al; 2009). Gunduz et al found that IMA increases in cerebrovascular diseases as the degree of oxidative stress increases even before AIS and may serve as a biomarker for early identification of acute cerebral ischemia (Gunduz et al; 2011). IMA may help in prediction and therapeutic decision making in management of AIS patients hence help reduce mortality rates (Abboud et al, 2007). IMA is not specific for cardiac or cerebral ischemia, it may also be elevated in most patients with inflammatory and oxidative stress-associated diseases such as liver cirrhosis, acute infections and advanced cancers; all these conditions are potent enhancers in the production of free radicals (Mentese, et al; 2007).

II) Prognostic value of IMA:

IMA is increased significantly in acute ischemic stroke (AIS) patients on admission. (Gunduz et al, 2011). It has been reported that the IMA levels may return to normal levels within a few hours (12 to 24 hours) with removal of free radicals (Talwalkar et al, 2008). This has also been observed in follow up of AIS samples (at 24, 48 and 72 hrs) as compared to admission values.
The treatment regimens given to AIS patients like anti-platelets and anti-edema agents act as antioxidants, hence further decrease in IMA level in AIS samples could be the result of beneficial treatment procedure. (Cuzzocrea et al, 2001). Sameer and colleagues showed that follow up estimations of IMA in AIS help in the prediction of the clinical status and outcome in AIS patients (Sameer Abboud et al; 2008). Although some studies have suggested that IMA is a poor predictor of adverse outcomes in the short term, this may be explained by taking into account that IMA elevation is non-specific and hence may attenuate any prognostic significance if it is used for prognosis before first establishing the diagnosis (Collinson et al, 2008).

2.2 Oxidative stress

In vivo, 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 like cobalt. (Roy et al; 2006). Oxidative stress is a strong underlying factor in hypertension (Ceriello et al; 2008) and results from increased ROS production and/or reduced antioxidant mechanisms. (Nickenig and Harrison et al; 2002). Oxidative stress is reported to be high in patients with hypertension as suggested by elevated biomarkers of oxidative stress such as 8-isoprostane and plasma malon-dialdehyde in these patients (Schiffrin et al; 2008). Increased oxidative stress is associated with decreased activity of antioxidant enzymes such as SOD, catalase and glutathione transferase and with increased activity of ROS generating enzymes. (Lee and Griendling et al; 2008). Oxidative stress modulates both vasoconstriction and dilation via generation of ROS that can directly act as signaling molecules and contribute to the pathophysiology of hypertension (Moraweitz et al; 2001)). The predominant enzymes involved in ROS generation in the vasculature are NADPH oxidase, uncoupled eNOS, xanthine oxidase and the mitochondrial electron transport system (Lee and Griendling, 2008). Oxidative stress has also been shown to uncouple endothelial nitric oxide synthase (eNOS) resulting in impaired endothelium dependant relaxations (Munzel et al; 2005). ROS including peroxynitrates can deplete intracellular tetrahydrobiopterin (BH4) by oxidative modification of BH4 to bihydrobiopterin (BH2) resulting in eNOS uncoupling ( Landmesser et al; 2003). When eNOS is uncoupled, electrons are directed towards molecular oxygen instead of L-arginine, generating
superoxides instead of NO (Munzel et al., 2005). Therefore, oxidative stress can contribute to endothelial dysfunction by scavenging NO and uncoupling eNOS. (Puddu et al; 2000).

2.3. Hypertension

Hypertension is a multifactorial disorder that affects at least 1 in 3 adults, and phenotypically manifested as a sustained elevation in arterial pressure (systolic pressure of 140 mmHg or higher and/or a diastolic pressure of 90 mmHg or higher). (Chobanian et al; 2003). Approximately 95% to 98% of the hypertension cases fall into the category termed as ‘Primary’ or ‘Essential’ hypertension i.e. no clear single identifiable cause is found for hypertension development in these patients (Cain et al; 2002). Despite significant advances in hypertension research, blood pressure is effectively controlled in only about 31% of the hypertensive patients (Arguedas et al; 2009).

The risk for fatal and non-fatal cardiovascular diseases is lowest in adults with blood pressure lower than 120/80 mmHg, risk increase significantly with elevation in blood pressure above 120/80 mmHg (Diaz et al; 2014). The World Health Organization estimated that approximately 50% of cases of cardiovascular disease and 75% of strokes are caused by elevated blood pressure (WHO, GHO; 2015). Thus, high blood pressure poses a serious health care challenge and prevention or effective management of hypertension can significantly lower the prevalence of stroke and other serious cardiovascular and renal diseases (Arima et al; 2006).

Pathophysiology of Hypertension

Hypertension is a polygenic disorder whose pathogenesis involves multiple complex mechanisms that include but not limited to, sympathetic nervous system hyperactivity, renin-angiotensin-aldosterone system hyperactivity, defect in renal natriuretic and diuretic ability and structural and functional vascular changes. (Cain et al; 2002). According to Cain et al, these pathomechanisms of hypertension affect parameters that govern arterial blood pressure i.e. cardiac output, peripheral vascular resistance and blood volume.

I) Vascular Remodeling:
Most patients with essential hypertension exhibit normal cardiac output with sustained elevation in peripheral vascular resistance (Beevers et al; 2001). Thus, the hallmark hemodynamic change in hypertension is an increase in total vascular resistance secondary to altered vascular reactivity caused by structural changes (vascular remodeling) and/or functional changes (endothelial dysfunction) in the arteries resulting in enhanced vasoconstriction and/or impaired vasodilation. (Cain et al; 2002). Vascular remodeling is a major contributor to narrowing of arteries and thus increase in peripheral vascular resistance. It involves vascular smooth muscle cell proliferation, inflammation and cell migration and results in an increase in vessel wall thickness, reduced lumen diameter and increased media: lumen ratio (Touyz et al; 2003).

II) Endothelial Dysfunction:

Normal arterial pressure results from a dynamic equilibrium of counter-regulatory constrictor and dilator stimuli (Moraweitz H et al; 2001). In hypertension endothelial dysfunction causes a shift of the equilibrium towards enhanced vasoconstriction thereby increasing vascular resistance. (Puddu et al; 2000). Impairment of NO synthesis and/or bioavailability causes endothelial dysfunction and oxidative stress can contribute to endothelial dysfunction (Dudzinski et al; 2006). ROS, particularly superoxides, react rapidly with NO resulting in formation of peroxynitrite, thereby reducing NO availability and consequently reducing its biological activity (Ceriello A et al; 2008). Oxidative stress has also been shown to uncouple endothelial nitric oxide synthase (eNOS) resulting in impaired endothelium dependant relaxations (Munzel et al; 2005). ROS including peroxynitrites can deplete intracellular tetrahydrobiopterin (BH4) by oxidative modification of BH4 to bihydrobiopterin (BH2) resulting in eNOS uncoupling (Landmesser et al; 2003). When eNOS is uncoupled, electrons are directed towards molecular oxygen instead of L-arginine, generating superoxides instead of NO (Munzel et al; 2005). Therefore, oxidative stress can contribute to endothelial dysfunction by scavenging NO and uncoupling eNOS. (Puddu et al; 2000). It has been suggested that these functional changes may be the primary events contributing to the development of vascular remodeling and hypertension (Cain et al; 2002).

III) Renin-Angiotensin-Aldosterone System (RAAS):
RAAS plays a pivotal role in regulation of cardiovascular processes such as vascular resistance, salt and water homeostasis and tissue remodeling (Nickenig et al; 2002). The major physiological effector of RAAS is Angiotensin II (Ang II) which via endocrine, paracrine and autocrine mechanisms acutely causes potent vasoconstriction and regulation of sodium and water homeostasis, whereas chronic effects include vessel remodeling, renal fibrosis and cardiac hypertrophy (Mehta et al; 2007). Ang II is primarily synthesized via the classical or renal RAS and released into the circulation, nontraditional Ang II formation can also occur via non-ACE dependent pathways primarily mediated by chymase (Agarwal et al; 2013).

Figure 1: Pathway of the renin angiotensin aldosterone system (RAAS). (Agarwal et al; 201
CHAPTER THREE

METHODOLOGY

4.1 Study site and design

This analytical study involving adult hypertensive patients (with and without stroke) (cases) and normotensive (controls) was conducted at the University Teaching Hospital (UTH) under the department of Pathology and Microbiology in collaboration with the department of Internal medicine; Adult medical emergency unit (AMEU) at UTH.

4.2 Target population

Persons presenting to AMEU at UTH for medical services aged 25 – 70 years

4.3 Study population

All individuals who meet the inclusion criteria were enrolled into the study. A study control group of individuals that did not have a diagnosis of hypertension, chronic inflammatory condition such as rheumatoid arthritis and were not pregnant at the time of the research were recruited from health individuals that came to UTH for medical exams or volunteers. The cases and controls were matched for age and sex to reduce bias.

4.4 Sample size

A total sample size of 63 participants (21 hypertensive’s, 21 hypertensive’s + stroke and 21 non-hypertensive’s) were enrolled as calculated using the formula for determination of sample size for comparative research studies between groups as given below;

\[
N = \frac{6 \times 10^2(1.960 + 1.282)^2}{10^2}
\]

\[
N = 63.063384
\]

Thus  \( N = 63 \)
Where; N is the total sample size (the sum of the sizes of the comparison groups), \( \sigma \) is 10; the assumed SD of each group (assumed to be equal for groups), the \( z_{crit} \) value is 1.960 as given in tables for Standard Normal Deviate (\( z_{crit} \)) corresponding to the desired significance criterion of 0.05 or 95% confidence interval (CI), the \( z_{pwr} \) value is 1.282 as given in Standard Normal Deviate (\( z_{pwr} \)) tables corresponding to 90% statistical power, and \( D \) is the minimum expected difference between the means which has been estimated at 10. Both \( z_{crit} \) and \( z_{pwr} \) are cut-off points along the x-axis of a standard normal probability distribution that demarcate probabilities matching the specified significance criterion and statistical power, respectively. The groups that make up N are assumed to be equal in number, also that the outcome variable of a comparative study is a continuous value for which means are compared, and ANOVA statistical analysis was used (Eng, 2003).

4.5 Sampling method(s)

Systematic sampling in which consecutive individuals with hypertension reporting to AMEU and found to meet the inclusion criteria (given below) were enrolled into the study sample. At least 4mls of blood sample was collected from each participant into a 4mls lithium heparized vacutainers and transported to the laboratory where it was processed and stored at a temperature of \(-20^\circ C\) to be analyzed on a later date. The control group was selected by means of frequency matching of the same proportional characteristics (age and sex) as the study sample.

4.6 Case definition

Hypertensive patients were considered to be: Those with a mean daytime blood pressure values of 140 mmHg systolic or of 90 mmHg diastolic or greater. Categories of hypertension based on blood pressure values:

Inclusion Criteria:

- Individuals with elevated blood pressure that could not be attributed to the conditions included in the exclusion criteria (whether on antihypertensives or not) at the time of the study.
- Individuals aged 18 years and above.
- Individuals with stroke secondary to hypertension (i.e. stroke + hypertension).
- Individuals who had read (or read to) and understood the rationale of participating in the research.
- Individuals who gave written personal consent.

Exclusion criteria

- Individuals known to be hypertensive whose blood pressure was normal at the time of the study.
- Patients who had liver, any form of kidney dysfunction or have any form of cancer or any acute or chronic inflammatory disease, as determined by a leukocyte count of greater than 11 x 10^9 cells per litre, or clinical signs of infection.
- Individuals who have undergone any major surgery within the past one month.
- Non-Negroid Zambians.
- Pregnant women.
- Non-consenting individuals

This information was obtained from the patient files and from the interviews administered using a questionnaire.

4.7 Data collection

Clinical Data and Demographic Data Collection

Participants were recruited at AMEU during normal clinic hours both day and night, from Monday to Saturday. The AMEU personnel were made aware of the study and requested they
inform and explain to the participants about the study as they attended to them and also provide the participants with the study information sheet (see annex 2). If a patient autonomously agrees to be part of the study, they were required to sign the consent form (see annex 3), and were assigned a serial number.

Thereafter information on the patient’s demographic data, medical history, and on the spot blood pressure were recorded and compiled using a questionnaire (see annex 4). The demographic data included the participants’ age, sex, occupation, marital status and weight. The medical history data included the specific year in which the participant was diagnosed with hypertension, past and current medication list and dosage, and the presence of medical condition that would confound the research findings such as major surgeries, chronic inflammatory conditions and cancer.

Blood Pressure Determination

The participant’s routine on the spot blood pressure was measured with a mercury sphygmomanometer in the sitting position after 5 minutes of resting in a quiet environment following the commendations by the British Hypertension Society. Mean of 3 readings of systolic blood pressure (SBP) and diastolic blood pressure (DBP) (Korotkoff phase I and phase V, respectively) was taken at 5 minutes interval. The blood pressure reading obtained was transcribed on to the questionnaire. The patients’ file was also reviewed to find any further relevant data to the research and also to confirm the accuracy of information provided by the participant and recorded on the questionnaire.

Specimen Collection

The researcher collected Blood samples from the conscious participants via venipuncture from the antecubital vein. 4mls of blood was collected in a 5ml syringe using 21G bore size needles. The collected blood sample was then be 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 30 minutes of collection for processing.
Anticipated study related injuries such as hemorrhage and pain from sample collection were treated by the involved nursing staff in AMEU or the appropriate person was contacted by the researcher depending on the type of injury.

Specimen Preparation and Storage

In the laboratory, each specimen serial number was recorded on to a compilation summary sheet. Part of the blood specimen was processed for routine laboratory tests such as 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 aliquoted and then stored in a freezer at -20°C until the specimens was required for IMA analysis.

4.8 Specimen analysis

Assessment of Ischemia Modified Albumin (IMA)

IMA was measured using the IMA ELISA assay. The assay is based on the fact that ischemia modified albumin 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. The IMA ELISA Kit, abx250303 used was purchased from Abbexa Limited, England.
Analysis of albumin, Urea, and ALT

These were determined using the Olympus AU 400 chemistry analyzer in the UTH Clinical Chemistry laboratory. Test kits used were procured by the research team. A duplicate copy of each test results for each patient was sent to the patients file for which the patient was later notified of their results by the clinician.

4.9 Quality control

To ensure reliable results, quality control was performed on all the analytical instruments and analyzers to be used for any purpose during specimen analysis according to the UTH quality control guidelines. Quality control included equipment calibrations and analytical control runs on every analyzer before each test analysis.

4.10 Statistical analysis

Data was analyzed using IBM SPSS Statistics version 23 for Windows and results summarized onto tables and graphs. The one way analysis of variance (ANOVA) test and the Student t test for independent variables was used to compare mean values of serum IMA concentration between the three groups (hypertensive’s, hypertensive’s with stroke and normotensives), and any other possible confounders. All statistical tests were performed at 95% confidence interval with p-value of <0.05 to determine statistical significance.

4.11 Ethical considerations and permissions

Patient information and results were confidential and access to this information was restricted to the researcher, supervisor and clinicians only. The questionnaire captured the participants’ serial number hence specimen tubes will be identified by serial numbers. The file number was obtained for the purpose of returning the results.

The study participants were provided with an information sheet and given a thorough explanation of intent and rationale of the research after which the patient gave written informed consent freely. All this was done in private on a one to one basis to avoid undue influence that may affect or substitute the patient’s will for that of any other persons.
The research proposal was submitted to the University of Zambia Biomedical Research Ethics Committee (UNZA-BREC) for approval. Permission to conduct the study was sought from the UTH medical superintendent, the Head of Department Internal Medicine, and the Directorate of Research and Graduate Studies (DRGS) through the Assistant Dean, Postgraduate.

Permission to use equipment such as the ELISA machine and other laboratory facilities in the clinical chemistry laboratory at the University Teaching Hospital (UTH) was obtained from the Head of the Department of Pathology and Microbiology at the University Teaching Hospital.

4.12 Expected outcome

The study was expected to provide baseline data on IMA levels of the 3 different study classes and how this varies with development of a complication like stroke. Overall, information obtained through this study was to provide novel knowledge pertaining to hypertension in Zambia, which may help improve the treatment and overall management of ischemia associated complications in hypertension. The generated data was also to provide a basis for further research on hypertension.
CHAPTER FOUR

RESULTS

A total of 63 participants were recruited into the study and their demographic distribution is shown in table 1. ANOVA showed there was a significant difference in mean serum IMA levels between 3 groups (Table 4). Student t test revealed that the difference between group 1 (Controls), group 2 (Hypertensive’s) and Group 3 (Hypertension with stroke) was significant ($p < 0.0002$), but among group 2 and group 3 was not significant ($t = 1.679$; Table 5). Table 6 showed that IMA had a diagnostic accuracy of 86%, a negative predictive value of 71%, positive predictive value of 97% and a sensitivity of 81% with specificity at 95% when used as a biomarker for determining oxidative stress levels in hypertensive patients. Table 7 showed that the IMA assay was a poor biomarker for use in discriminating hypertensive patients likely to develop stroke, with a diagnostic accuracy at 55%, a negative predictive value of 63%, a positive predictive value of 53% and sensitivity of 86% with specificity at 24%.

Table 1: Demographic Distribution of the study Participants

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Normotensive</th>
<th>Hypertensive without stroke</th>
<th>Hypertensives with stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>Female</td>
<td>10</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Male</td>
<td>11</td>
<td>9</td>
<td>18</td>
</tr>
<tr>
<td>Mean age</td>
<td>36</td>
<td>48</td>
<td>49</td>
</tr>
</tbody>
</table>

*Total of 63 participants were recruited, 25 females and 38 males.*
Table 2: IMA mean serum levels (ng/mL)

<table>
<thead>
<tr>
<th></th>
<th>Normotensive</th>
<th>Hypertensive without stroke</th>
<th>Hypertensive with stroke</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.388</td>
<td>10.997</td>
<td>11.139</td>
</tr>
<tr>
<td>2</td>
<td>6.242</td>
<td>7.984</td>
<td>9.483</td>
</tr>
<tr>
<td>3</td>
<td>6.373</td>
<td>11.652</td>
<td>13.015</td>
</tr>
<tr>
<td>4</td>
<td>6.323</td>
<td>9.752</td>
<td>11.393</td>
</tr>
<tr>
<td>5</td>
<td>6.849</td>
<td>10.736</td>
<td>11.296</td>
</tr>
<tr>
<td>6</td>
<td>6.228</td>
<td>8.884</td>
<td>12.3</td>
</tr>
<tr>
<td>7</td>
<td>7.381</td>
<td>10.703</td>
<td>11.011</td>
</tr>
<tr>
<td>8</td>
<td>6.228</td>
<td>11.227</td>
<td>12.503</td>
</tr>
<tr>
<td>9</td>
<td>6.52</td>
<td>10.837</td>
<td>9.832</td>
</tr>
<tr>
<td>10</td>
<td>6.725</td>
<td>9.807</td>
<td>9.552</td>
</tr>
<tr>
<td>11</td>
<td>6.378</td>
<td>10.61</td>
<td>10.849</td>
</tr>
<tr>
<td>12</td>
<td>6.432</td>
<td>8.964</td>
<td>8.987</td>
</tr>
<tr>
<td>13</td>
<td>6.259</td>
<td>8.764</td>
<td>10.389</td>
</tr>
<tr>
<td>14</td>
<td>6.338</td>
<td>10.866</td>
<td>8.607</td>
</tr>
<tr>
<td>15</td>
<td>6.474</td>
<td>9.877</td>
<td>12.322</td>
</tr>
<tr>
<td>16</td>
<td>6.18</td>
<td>11.187</td>
<td>11.205</td>
</tr>
<tr>
<td>17</td>
<td>6.478</td>
<td>10.259</td>
<td>8.726</td>
</tr>
<tr>
<td>18</td>
<td>8.212</td>
<td>9.799</td>
<td>10.952</td>
</tr>
<tr>
<td>19</td>
<td>7.019</td>
<td>8.727</td>
<td>9.945</td>
</tr>
<tr>
<td>20</td>
<td>6.938</td>
<td>10.166</td>
<td>10.827</td>
</tr>
<tr>
<td>21</td>
<td>9.225</td>
<td>11.359</td>
<td>10.812</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>MEAN</th>
<th>SD</th>
<th>P value when compared to normotensives</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive</td>
<td>6.723</td>
<td>0.733</td>
<td>P &lt; 0.0002</td>
</tr>
<tr>
<td>Hypertensive without stroke</td>
<td>10.15</td>
<td>0.98</td>
<td>P &lt; 0.0002</td>
</tr>
<tr>
<td>Hypertensive with stroke</td>
<td>10.721</td>
<td>1.211</td>
<td>P &lt; 0.0002</td>
</tr>
</tbody>
</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.

Figure 2: The graph revealed a noticeable differences of the mean serum IMA levels of the normotensives from the other 2 groups, while difference between hypertensives and hypertensives with stroke was only slight. The statistical significance of the observed differences where determined in the calculation of ANOVA and the student t test below.

Table 3: Statistical parameters used to generate test statistics for ANOVA table and students t test

<table>
<thead>
<tr>
<th></th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>Hypertensive with stroke</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>∑ X</td>
<td>141.19</td>
<td>213.157</td>
<td>225.145</td>
<td>579.492</td>
</tr>
<tr>
<td>∑ X²</td>
<td>960.469</td>
<td>2183.635</td>
<td>2444.539</td>
<td>5588.643</td>
</tr>
<tr>
<td>N</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
</tr>
</tbody>
</table>
### Table 4: ANOVA parameters

<table>
<thead>
<tr>
<th>Source</th>
<th>Df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between</td>
<td>2</td>
<td>196.371</td>
<td>98.186</td>
<td>85.259</td>
</tr>
<tr>
<td>Within</td>
<td>60</td>
<td>61.939</td>
<td>1.032</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>258.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

At a level 0.05 and degree of freedom 2 and 60, $F$ table critical value = 3.15. Our calculated $F$ value was 85.259, which was larger than the table critical value (3.15). Thus there was a significant difference in the serum IMA levels between the 3 groups. This was due to the high degree of oxidative stress in the hypertensives participants.

### Table 5: Student t test results for IMA differences between 2 groups

<table>
<thead>
<tr>
<th>Group</th>
<th>t-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA level of normotensives and hypertensive’s</td>
<td>12.833 ($P &lt; 0.0002$)</td>
</tr>
<tr>
<td>IMA level of hypertensives and hypertensives with stroke</td>
<td>1.679</td>
</tr>
</tbody>
</table>

At a 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 a level 0.05. Thus IMA could not distinguish between hypertension only and hypertension with stroke.
Table 6: IMA test performance for predicting oxidative stress in hypertensives at serum IMA cut off level of 9 ng/mL

<table>
<thead>
<tr>
<th>Clinical condition status</th>
<th>hypertensives</th>
<th>normotensives</th>
<th>Total tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA test positive</td>
<td>34</td>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>IMA test negative</td>
<td>8</td>
<td>20</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>42</td>
<td>21</td>
<td>63</td>
</tr>
</tbody>
</table>

From the table above IMA assay sensitivity is 81%, specificity is 95%, positive predictive value is 97% and negative predictive value is 71% and a test diagnostic accuracy of 86%.

Table 7: IMA test performance for predicting likelihood stroke in hypertensives at serum IMA cut off point of 9 ng/mL

<table>
<thead>
<tr>
<th>Clinical condition status</th>
<th>Stroke present</th>
<th>Stroke absent</th>
<th>Total tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA test positive</td>
<td>18</td>
<td>16</td>
<td>34</td>
</tr>
<tr>
<td>IMA test negative</td>
<td>3</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>21</td>
<td>21</td>
<td>42</td>
</tr>
</tbody>
</table>

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%.
CHAPTER FIVE

DISCUSSION

Stroke is a well recognized complication of hypertension and is arguably one of the leading cause of morbidity and mortality in developed and developing countries of the world. (2013 American Heart Association publication, 16th October). Increased oxidative stress is one of the principal mechanisms by which hypertension exerts its pathological influence leading to complications such as stroke. (Cachofeiro et al, 2009). IMA assay is a sensitive marker for early detection of stroke and levels tend to be higher in ischemic stroke than in hemorrhagic stroke. (Sameer et al., 2008). IMA is still a relatively new biomarker on the market with a great deal of information yet to be known about it, thus it is 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 (Aslan et al; 2005). Our study showed that IMA had a negative predictive value of 71%, a positive predictive value of 97% and diagnostic accuracy of 86%, with a sensitivity and specificity of 81% and 95% respectively at an IMA cut off value of 9 ng/mL. These figures are above the 70% mark and thus IMA could be utilized as a biochemical test to evaluate the extent of ongoing ischemia resulting from endothelial dysfunction secondary to oxidative stress in a hypertensive patient more especially if there are no overt clinical features. The obtained IMA value should be interpreted with comparison to the measured blood pressure i.e. high IMA value in a patient with very high blood pressure means increased likelihood of hypertensive complication, while a low IMA means lesser degree of oxidative stress and thus ongoing ischemia, hence low likely hood of complication. However these predictive values needed to be interpreted in conjunction with existing clinical features of the patient. (Collinson et al, 2006).

Our results showed an increased IMA levels in hypertensives and hypertensives with stroke compared to the normotensive 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
The increased IMA level in the hypertensive groups was most likely due to increased oxidative stress in the hypertensive’s whether with stroke or not. (Sameer Abboud et al; 2001). 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. (Bar-Or D et al; 2001). This reduced binding of transitional metals causes release of more Cu²⁺, thus generating more free radicals through the Fenton reaction. The resulting vicious cycle induces a sudden increase in IMA (Roy et al; 2006). 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 (Ahn et al; 2011).

This 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 literature reviewed that IMA is a non specific indicator of oxidative stress (Mentese et al; 2008), but it could also be inferred from these results that probably the correlation between hypertension, oxidative stress and the occurrence of stroke is not as simple as it was thought to be. Other factors that could explain our observations were found in the research by Gunduz et al in 2011. 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, 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 (Aslan et al; 2005). This 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 consistent with previous studies that IMA is a non specific biomarker of oxidative stress. (Mentese et al; 2008). The results may also suggest that the stroke may be associated with hemorrhagic episodes rather than ischaemic episodes in our Zambian patients, and the lesions may be stabilized with alternative blood supply to the ischaemic area. Thus, it would be ill advised to use IMA as a stand alone 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.
CHAPTER SIX

CONCLUSION AND RECOMMENDATIONS

7.1 Conclusion

We demonstrated raised serum IMA levels in hypertensives (both with and without stroke) compared to normotensives signifying increased oxidative stress in the hypertensive participants. However statistically similar serum 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.

7.2 Recommendations

IMA is a relatively a new bio-marker with huge potential for application in management of ischemia associated organ dysfunction such as ischemic stroke. Of all the suggested biomarkers that are said to detect ischemia way before infarction occurs IMA currently remains the only ischemia assay to have reached the clinical validation stage. For efficient provision of care in the Emergency Department, a high negative predictive value may be critical, because the correct exclusion of ischemia before infarction preserves limited and expensive resources, such as stress tests, hospital beds and catheterization slots. Use of IMA in such a manner as a tool to help forestall hypertension associated ischemia complications through early intervention may in the long term be more cost effective than treating the said complications and their effects when they occur. The cost of managing these complications is not just in monetary form but also in the form of both physical and mental toll it takes on the family members and community caring for the patient. However little is known about this novel bio-marker and thus extensive research into IMA is needed especially for the following:

1. Since susceptibility of the cells to ischemia may vary from one organ to another, it would be critical to determine the optimal IMA level for diagnosis of ischemia in various organs especially the heart and the brain.
2. Extensive study with a larger patient population is required to compare the IMA test sensitivity and specificity to other markers and tests such as CT scan, to determine the use of IMA as an independent point of care test or could it just be used as an additional parameter to boost the confidence of the clinician in ruling out ischemia related organ dysfunction.

3. Need for more research into the use of IMA as an early marker for onset of hypertension.

4. Need for more research to be conducted especially on defining what a positive IMA might mean in terms of guiding therapy and predicting long term clinical outcome in patients with hypertension and stroke.

Shift in reimbursement would encourage hospital emergency rooms to use best practices such as IMA, to perform better risk stratification of patients up front before determining who requires inpatient care. Thus use of IMA tests and results in such a manner may help move patients into a low risk category by initial evaluation based on the clinical presentation, leading to a reduction in unnecessary hospital admissions and in the long run decongest the wards.

7.3 Limitations

However, as promising as the IMA assay appears some degree of caution is still indicated. The currently accepted strength of IMA lies in its negative predictive value for excluding the presence of ischemia (Aslan, Apple; 2005). It is a basic principle of diagnostic testing that adding tests together increases sensitivity. Thus, adding IMA to traditional markers will increase sensitivity, but so would adding other biomarkers (Bainbridge et al, 2006).

Limitations encountered during the study included:

- Unwillingness of people to participate in the study.
- Availability of resources to successfully complete the study as the test kits were expensive and the required sample size was relatively large.
- Inability to account for some of the possible confounding factors because of limited diagnostic facilities at the location the study was carried out.
- Inability to do CT scans to confirm if the clinically suspected cerebrovascular accident was indeed the ischemic type.
REFERENCES


APPENDICES

Annex 1: Information sheet

My name is Peter Simoonga, studying for a MSc. Pathology (Chemical Pathology) at the University of Zambia. I am carrying out a research (study) as a partial fulfillment of Master Degree. Please feel free to ask any question as I explain about the study.

About the Study:

The study will examine the levels of ischemia modified albumin (IMA) in the blood. Ischemia modified albumin is albumin that has undergone a change in structure because of reduced blood supply. Albumin is one of the major proteins in circulation in the body and has a lot of functions such as carrying metals and drugs to areas of the body where they are needed or for excretion.

During periods of reduced blood supply the albumin changes structure so as to help remove the harmful products generated during this period, this causes a reduction in albumin’s ability to bind some of the metals it normally binds. The degree to which this reduced binding occurs can be measured to give an approximation of the extent of ischemia. A reduction in blood supply to organs of the body is a common effect of hypertension and so we will attempt to measure this altered albumin in people with hypertension and compare it to those without hypertension in an effort to generate information that may help in improving the treatment of hypertension.

Participating in this Study:

You are invited to take part in this research study. You can decide whether you want to join this study or not. You are free to say yes or no. And even if you join this study, you do not have to stay in it. You may stop at any time without penalty. If you decide to take part in the study, you will be asked a few questions which you may not answer if through your own understanding seem personal, uncomfortable or otherwise. You will also be requested to give 4mls of blood that shall be collected by the researcher or the nurse. This blood is needed because it will be used to measure the altered albumin. The study requires at least 63 people to participate.
You have been invited to participate in this study because you are an adult with hypertension and also based on the clinician’s assessment with regard to the study inclusion criteria as administered in the study questionnaire. (You have been invited to participate in this study because you are adult presumed **NOT to have** hypertension, and also based on the clinicians’ assessment with regard to the study inclusion criteria as administered in the study questionnaire)*.

**Benefits for participation:**

There are no costs to you for being in this study and the study may not benefit you directly. However the study results will provide valuable information about your health and this information will be made available to through your physician. It is hoped that information generated through this study as a result of your participation will help medical personnel to better understand hypertension, and thus help improve management and treatment. Thus your participation in this study may help thousands of people in many years to come.

**Problems with the study:**

The problems that some people have experienced in studies like this are some pain when blood is being drawn from the vein or loss of confidentiality. Every effort will be made to reduce the pain that you feel as the blood is collected and also to keep your personal medical records confidential. It is also important to understand that obtaining a blood sample from some of the participants may be more difficult than from others.

Apart from the problems mentioned above, blood collection from healthy, non-pregnant adults who weigh at least 50 kilograms poses minimal risk. Other risks associated with having blood drawn are slight but may include; Excessive bleeding, fainting or feeling light-headed, blood accumulating under the skin, infection(a slight risk any time the skin is broken).

• All study related injuries shall be treated by the nurses and doctors with the UTH Out-Patient Medical Clinic.

**Contact Details:**
In case you have any more questions about this study at any time; you may call any of the numbers below;

1. **Peter Simoonga**

The Researcher
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2. **Prof. Trevor Kaile** (Principle Supervisor)

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3. **The Chairperson**

University of Zambia Biomedical Research Ethics Committee (UNZABREC)
Ridgeway Campus
P.O Box 50110
Lusaka, Zambia
Contact Number: 260-1-256067
Email Address; unzarec@zamtel.zm
Annex 2: THE INFORMED CONSENT FORM TO PARTICIPATE IN THE STUDY

Study Title: ASSESSMENT OF ISCHEMIA MODIFIED ALBUMIN (IMA) AS A BIOMARKER OF OXIDATIVE STRESS IN SUSPECTED SUBCLINICAL ISCHEMIC EPISODES IN HYPERTENSIVE BLACK ZAMBIANS AT THE UNIVERSITY TEACHING HOSPITAL.

By signing my name below, I .............................................................. confirm the following:

- I have read (or had read to me) this entire consent document and all of my questions have been answered to my satisfaction.
- The study's purpose, procedures, risks and possible benefits have been explained to me.
- I agree to let the study team use and share the health information and other information gathered for this study.(the participant will not be personally identified).
- I voluntarily agree to participate in this research study and I agree to give small amount (4mls) of blood.

**Participant signature**............................... **Date**.................................

Thump Print in This box

You (the participant) will receive a signed and dated copy of this consent form. Please keep it where you can easily find it. It will help you remember what we discussed today.

Statement by the researcher/person taking consent:

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the following:

- The research procedure.
They are free to skip questions they may deem personal or otherwise.

They are free to withdraw from the study at anytime without penalty.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Name of Person Taking Consent: __________________________ Signature: __________

Name of witness: ________________________________ Signature: ______________

Date ___________________________ Day/month/year

A copy of this ICF has been provided to the participant.
Annex 3: HYPERTENSION RESEARCH QUESTIONNAIRE

THE UNIVERSITY OF ZAMBIA

PATHOLOGY AND MICROBIOLOGY DEPARTMENT

This questionnaire is administered for;

A STUDY TO INVESTIGATE THE SERUM LEVELS OF ISCHEMIA MODIFIED ALBUMIN IN HYPERTENSIVE BLACK ZAMBIANS

INSTRUCTIONS;

- Ensure you put the answers in the space provided and in legible writing.
- If the provided space is not sufficient, you may include additional paper where necessary.
- In the event you make a mistake please cancel the mistake neatly and provide the correct answer just next if space allows, otherwise refer to point number 2.
- Where the question is not applicable please indicate with ‘NA’.

Date………………………….

PATIENT ID………………………………………………… SERIAL #…………………..

Sex.......................... Age........................... Phone #.............................................

Weight.................. Height.................. BMI..............................

DEMOGRAPHIC DATA

a) Marital Status............... b) Occupation.................. d) Residence.....................

MEDICAL HISTORY AND GENERAL HEALTH

a) When were you diagnosed with HYPERTENSION? .................. (Indicate Years Since)
b) Have you been told what may have caused your hypertension?...............(if yes, please specify)

b) Are you on any antihypertensive medication? ........................................... (Indicate Drug Name)

c) have you ever been admitted because of a hypertension related complication?...............(if yes please give details e.g. what complication and how many times)

c) Any family history of hypertension?................................(if yes please specify)

d) Do you have any (other) chronic conditions?

d) Have you had any surgery in the past 2 months?......................... (If yes specify the indication)

f) Do you smoke cigarettes?

PHYSICAL ACTIVITY


b) How heavy do you exercise?

DIET

Type: a) High fat……………. b) High protein…………….. c) High carbohydrate………………

d) Do you add a lot of salt to food? ………………………………………………………………….

e) Last Meal: How long ago was the last meal?
(hours)…………………………………………………………

d) What did you eat in last meal………………………………………………………………………

Thank you for your help!