EVALUATION OF SERUM LEVEL OF HOMOCYSTEINE, VITAMIN B_{12} AND ZINC IN PATIENTS WITH ACUTE ISCHAEMIC STROKE IN ZARIA

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MAY, 2015

DECLARATION

I declare that the work in this dissertationentitled **Evaluation of serum levels of homocysteine, vitamin B_{12} and zinc in acute ischaemic stroke patients in Zaria,** has been carriedout by me in the Department of Chemical Pathology, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

Hafsatu Maiwada Suleiman	………………………	………………………………
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled; EVALUATION OF SERUM LEVELS OF HOMOCYSTEINE, VITAMIN B_{12} AND ZINC IN ACUTE ISCHAEMIC STROKE PATIENTS IN ZARIA byHafsatu MaiwadaSULEIMAN meets the regulations governing the award of the degree of MSc Chemical Pathology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literacy presentation.

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ABSTRACT

Stroke has been a global burden, with increasing morbidity and mortality. Several risk factors have been identified, which include: hyperhomocysteinaemia, hypovitaminosis $B_{12,}$ and low zinc levels, which are the now target of preventive strategies. Limited studies have been done on the risk factors (analytes) in our environment hence the current study was undertaken to evaluate the serum levels of homocysteine, vitamin B_{12} and zinc in patients with acute ischaemic stroke in Zaria and healthy controls. One hundred ischaemic stroke patients on admission confirmed by brain CT-scan or Siri-raj stroke score of less than minus one.(-1) and equal number of apparently healthy age and sex-matched were recruited. Their serum homocysteine, and vitamin B_{12} were measured using enzyme linked Immunosorbent assay,and zinc was measured using direct colorimetric method. Stroke severity was determined using National Institute of Health Stroke Score (NIHSS). Mean serum homocysteine for patients was significantly higher than that of controls ($p<0.05$) and mean serum vitamin $B_{12}$and zinc were significantly lower compared to that of controls ($p<0.05$), with an odds ratio of 0.04, 0.19 and 0.54 respectively. The reference intervals obtained from the healthy controls were found to be 0.90 -1.70µmol/l, 199.72-685.48pg/ml and 52.26-111.86µg/dl for homocysteine, vitamin B_{12} and zinc respectively. Hyperhomocysteinaemia was seen in 34%, hypovitaminosis B_{12} was seen in 81% and low zinc was seen in 46%. Patients with hyperhomocysteinaemia, hypovitaminosis B_{12} and low zinc presented with more severe neurologic deficits even though the difference was not statistically significant with p-values of 0.946, 0.735, and 0.566 respectively. Elevated serum homocysteine, low vitamin B_{12} and zinc were found to be associated with ischaemic stroke. There was negative correlation between homocysteine and vitamin B_{12} and stroke severity and therefore early

management of those conditions may be an effective way of decreasing the incidence of stroke in our environment. Vitamin B_{12} and zinc supplements may be beneficial to patients at risk.

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ABBREVIATIONS AND SYMBOLS USED

% Per cent

< Less than

> Greater than

± Plus or minus

≤ Less than or equal

≥ Greater than or equal

µL Micro litre

µmol Micromole

µmol/L Micromole per litre

AAbsorbance

ABUTHAhmadu Bello University Teaching Hospital

ACA Anterior Cerebral Artery

AD Alzheimer's disease

AICA Anterior Inferior Cerebellar Artery

AMPA α-amino-3-hydroxy-5-methyl-4-Isoxanole propionate

Apo CI Apo Lipoprotein CI

Apo CIII Apo Lipoprotein CIII

APOE Apolipoprotein E gene

$A_{std:}$ Absorbance of standard

BP Blood pressure

C Concentration

CBS Cystathionine B-synthase

$-CH_2-$: Methylene bridge

CIM T Carotid intimal medial thickness

cInICardiac Troponin I

CK-MB Creatine kinase (MB fraction)

CRP C- Reactive Protein

CT Computerized tomography

cTnTCardiac Troponin T

DBP Diastolic blood pressure

DM Diabetes Mellitus

e.gFor example

ECG Electrocardiography

ELISA Enzyme-linked immunosorbent assay

et al And others

GFAP Serum glial fibrillary acidic protein.

GFR Glomerular filtration rate

HcyHomocysteine

HIV Human immunodeficiency virus

ICH Intracerebral Haemorrhage

LDH Lactate dehydrogenase

MCA Middle Cerebral Artery

minMinute

mlMilliliter

mmol/LMilli mole per litre

MR Magnetic Resonances

MTHFR C677TMethylenetetrahydrofolate reductase at position 677

MTHRF Methylene tetrahydrofolate reductase

ngNanograms

NIHSS National Institutes of Health Stroke Scale

NMDA N-methyl-D-aspartate

PAI-1 Plasminogen Activation Inhibitor -1

PCA Posterior Cerebral Artery

PICA Posterior Inferior Cerebellar Artery

rpmRevolution per minute

SAA Serum Amyloid A

SAM*S*-adenosyl methionine.

SBP Systolic blood pressure

SD Standard deviation

SPAF Stroke Prevention in Atrial Fibrillation

SPSS Statistical Package for Social Sciences

SWD Spontaneous Waves of Depolarisation

THFTetrahydrofolate

TIA Transient Ischaemic Attack

TPA Tissue Type Plasminogen Activation

uMMAUrinary methylmalonic acid

US: United States of America

VDRL Venereal disease research laboratory test

WHOWorld Health Organization

Wt Weight

yr Year

α	Alpha
β	Beta

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND

Stroke is defined as a clinical syndrome of sudden onset of rapidly developing symptoms or signs of focal and at times global loss of cerebral function, with symptoms lasting more than 24 hours or leading to death, with no apparent cause other than that of vascular origin (Kameshwar*et al*, 2012).

The World Health Organization estimates that cardiovascular disease and stroke will be the leading cause of death and disability world wide by 2020 (Lynn,2000). Stroke is one of the leading causes of death in any population, and its prevention is a key strategy in reducing the rate of mortality and morbidity (Hoseinali*et al*, 2011). It is the third commonest cause of death in Western industrialised countries (James *et al*,2000). Stroke is presently the leading cause of disability and the third leading cause of death in United States (US centers, 2007). In the United States, blacks have an age-adjusted risk of death from stroke that is 1.49 times that of whites (Schneider *et al*, 2004). More than 700,000 persons per year suffer a first time stroke in the United States with 20% of these individuals dying within the first year after stroke (American Heart Association, 2002).If current trend continues, this number is projected to reach one million per year by the year 2020(Ralph *et al*, 1997). In low income and middle income countries, the burden of stroke and other vascular diseases is likely to increase substantially over time in the next few decades because of their expected health and demographic transition (Ralph*et al*, 1997).

Globally in 2005, it was estimated that stroke caused 5.7 million deaths, and 87% of these occurred in low income and middle-income countries of the world (Strong *et al*, 2007)

Nigeria, the most populous black nation in the world (Kolawole, 2008), stands the risk of further straining of its resources as a result of the increasing prevalence of stroke and other non-communicable diseases due to epidemiological transition(Kolawole *et al*, 2008). The current prevalence of stroke in Nigeria is 1.14 per 1000 while the 30-day case fatality rate is as high as 40% (Kolawole *et al*, 2008). In Sokoto it was established that the 24 hour and 30 day case fatalities of stroke were 11.9 and 38.4 respectively (Abubakar *et al*, 2010). Management of the disease is largely conservative while there is little or no funding for quality research (Kolawole, *et al* 2008).

Several risk factors for stroke have been identified, which are the target of both primary and secondary preventive strategies (Hoseinali*et al*, 2011), these risk factors include hypertension, diabetes mellitus, cardiac diseases, sickle cell anaemia, cigarette smoking, other immerging or noble risk factors include hyperhomocysteinaemia, hypovitaminosis $B_{12,}$and low zinc levels etc. The role of hyperhomocysteinaemia as it relates to stroke in Africans is still uncertain. It was hypothesized that homocysteine levels are significantly higher in stroke patients than in normal controls and worsen stroke severity, and increase short-term case fatality rates following acute ischaemic stroke(Okubadejo*et al*,2008)

Homocysteine is an amino acid. It is a homologue of the amino acid cysteine, differing by an additional methylene bridge (-CH_2-). It is biosynthesized from methionine by the removal of its terminal C methyl group. Homocysteine can be recycled into methionine or converted into cysteine with the aid of B-vitamins (Wikipedia, 2013).

An abnormally high level of homocysteine in the serum, above the upper limit of an environment, constitutes hyperhomocysteinaemia. This condition is a significant risk factor for the development of a wide range of cerebrovascular diseases including stroke(Wikipedia, 2013). Deficiencies of vitamin B_{12} (cobalamin) can lead to high homocysteine levels and supplementation with B_{12} reduces the concentration of homocysteine in the bloodstream(Wikipedia, 2013).

Hyperhomocysteinaemia is a potentially modifiable risk factor for stroke, and may have a negative impact on the course of ischaemic stroke (Okubadejo*et al*, 2008).

The accumulation of homocysteine and its metabolites is caused by disruption of any of the 3 interrelated pathways of methionine metabolism deficiency in the cystathionine B-synthase (CBS) enzyme, defective methylcobalamin synthesis, or abnormality in methylene tetrahydrofolate reductase (MTHFR) (Pitchaiah, 2013)

Hyperhomocysteinaemia may cause endothelial dysfunction through oxidative stress, resulting in local thrombosis and subsequent ischemia. Another possible mechanism is the direct toxicity of homocysteine to blood vessels but there is no definite evidence to support either of these mechanisms(Hoseinali*et al*, 2011).

Vitamin B_{12}, also called cobalamin, is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of erythrocytes. It is one of the B vitamins.A study published by Eric et al using a highly accurate screening method called the urinary methylmalonic acid (uMMA) test identified undiagnosed B_{12} deficiency in the study's participants , this undiagnosed group was 2.6 times as likely to suffer a stroke(Strokes, 2014).

Zinc is one of the most abundant trace elements in the body second only to iron, it mediates several vital physiological functions and is essential for maintaining a healthy immune system and meeting metabolic demands. However, whether zinc exerts neuroprotective or neurotoxic effects during cerebral ischaemia is still unclear (Archit*et al*,2010). Low serum zinc concentrations are associated with more severe strokes on admission (Archit*et al*,2010).

1.2 STATEMENT OF THE PROBLEM

Stroke is one of the leading causes of death in any population. Serum homocysteine levels are reported to be elevated in patients admitted with acute ischaemic stroke while those of vitamin B_{12} and zinc were found to be lower compared to healthy subjects. These abnormalities indicate more severe strokes in some patients. Prevention of hyperhomocysteinaemia, hypovitaminosis B_{12} and low zinc may be a key strategy in reducing the rate of morbidity.

1.3 JUSTIFICATION

There has been reported increased morbidity and higher mortality with poor prognosis in acute ischaemic stroke patients with high homocysteine, low vitamin $B_{12}$and zinc compared to acute ischaemic stroke patients without these abnormalities. Even though there is large body of evidence in western literature that has linked admission serum homocysteine vitamin B_{12} and zinc and stroke severity and could be used as a marker of prognostication. There is paucity of information on serum homocysteine, vitamin B_{12} and zinc in acute stroke patients in sub-Saharan Africa and Nigeria in particular. This study is therefore aimed to add to existing body of knowledge on patients in admission serum homocysteine, vitamin B_{12} and zinc as they affect presentation, severity and outcome of acute ischaemic stroke patients in Zaria, Nigeria.

1.4 AIM AND OBJECTIVESOF THE STUDY

1.4.1 Aim

To evaluate at the time of admission the serum levels of homocysteine, vitamin B_{12} and zinc in patients presenting with acute ischaemic stroke seen in ABUTH Zaria.

1.4.2 Objectives

1. To measure serum levels of homocysteine, vitamin B_{12} and zinc in patients admitted with acute ischaemic stroke from January to December 2013 in ABUTH, Zaria.
2. To determine the prevalence of abnormalities of serumhomocysteine, vitamin B_{12} and zinc in patients with acute ischaemic stroke in ABUTH Zaria.
3. To compare the mean values ofhomocysteine, vitamin B_{12} and zinc among patients and healthy controls.
4. To determine the relationship between stroke severity and admission serum levelsof homocysteine, vitamin B_{12} and zinc.

1.5 RESEARCH QUESTION/HYPOTHESIS

The research question is whether serum levels of homocysteine is high and that of vitamin B_{12} and zinc is actually low in patients with acute ischaemic stroke than normal non stroke individuals and if those analytes really determine severity of the stroke. The null hypothesis therefore is serumlevels of homocysteine, vitamin B_{12} and zinc are not different from normal healthy non stroke individuals. The alternative hypothesis is serum homocysteine level is higher while serum levels of vitaminB_{12} and zinc are lower in patients with acute ischaemic stroke than normal healthy non stroke individuals.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 STROKE

4.1.1 Brief History

Stroke was first recognised over 2400 years agodescribed as the phenomenon of sudden paralysis and coined the term “cerebral apoplexy”, meaning “struck down with violence”

In 1599 the term “Stroke” was first used as a fairly liberal translation of the Greek “Struck down”. The Germans and French adopted similar translations ‘Schlag’ and ‘Coup’, respectively (Eric, 2011). It was not until 1658 when it was discovered that people died with stroke as a result of a disruption of blood flow to the brain and described cases of paralysis arising from cranial bleeding” (Eric, 2011) .

In 1730 progress in stroke research was temporarily derailed by Herman Boerhaaves lectures linking stroke to cacochymialeucophlegmatica, a pathologic excess of white phlegm (Eric, 2011). In 1847 a German pathologist, established atherosclerosis and clotting as definitive causes of ischaemic stroke (Eric, 2011).

In the 1970s population studies concluded that hypertension was the most common modifiable risk factor for stroke (Eric, 2011). A decade later cigarette smoking was clearly linked to stroke (Eric, 2011).Today there is a wealth of information available on the cause, prevention, risk and treatment of stroke.

2.1.2 Epidemiology

It is almost 2400 years since stroke was identified (Eric, 2011). Studies revealed that an estimated 16 million first time stroke patients and 5.7 million stroke deaths, accounting for nearly 10% of all deaths worldwide (Oni *et al,* 2009). It affects blacks more frequently than whites (Eric, 2011) and also affects more men than women, but women are more likely to die from stroke (Salvador, 2011). Despite being the second leading cause of death worldwide, funding for stroke research lags far behind funding for other major causes of death in Europe (Oni *et al,* 2009).

Stroke kills about 150,000 Americans each year which constitutes about one third of the stroke victims (Eric, 2011, Stroke, 2013). Three million Americans are currently permanently disabled by stroke (Stroke, 2013).

There are around 1.2 million stroke survivors in the UK and more than half suffer from post stroke disabilities that affect their daily life (Stroke Association, 2013). Every year an estimated 152,000 people in the UK have a stroke and can affect any age group, (Stroke Association, 2013). Around a third of all strokes occurred under the age of 65 (Stroke Association 2013). In Africa, stroke accounts for 0.9% to 4.0% of all hospital admissions, and 2.8% to 4.5% of total deaths (Oni*et al,* 2009). Incidence of stroke in Africa is on the increase (Oni*et al*, 2009)and Stroke causes a great burden on the family and the society at large due to prolonged hospital admission with subsequent disability (Oni*et al,* 2009). Stroke is the third leading cause of death in West Africa and a growing non-communicable disease in Nigeria (Ezealaadikube*et al,* 2010). It also accounts for 4.5-17% of all admission in hospital based studies (Kolawole*et al,* 2008).A crude prevalence rate of 1.31/1000 population was recorded at Kwara state. Two studies reported a stroke prevalence of 0.58/1000 and 1.14/1000 mixed urban community in south western Nigeria (Danesi*et al,* 2007,Onwuchekwa, 2014).

In Nigeria stroke accounts for 0.9-4.0% of medical admissions and 0.5-45% of neurological admission and the prevalence of stroke among women with DM is put at 0.9% (Ezealaadikube*et al*, 2010).

2.1.3 Anatomy of the Brain

2.1.3.1 Gross

The brain is the most metabolically active organ in the body. It represents 2% of body mass and requires 15-20% of the total resting cardiac output to produce the necessary glucose and oxygen for its metabolism. Knowledge of cerebrovascular arterial anatomy and the territories supplied by each is useful in determining which vessel is involved in acute stroke (Salvador, 2011).

2.1.3.2 Arterial Distribution

The cerebral hemispheres are supplied by three paired major arteries, the anterior, middle and posterior cerebral arteries.

The anterior and middle cerebral arteries arise from the supraclinoid internal carotid arteries. The anterior cerebral artery (ACA) supplies the medial portion of the frontal and parietal lobes and anterior portions of basal ganglia and anterior internal capsule. The middle cerebral artery(MCA) supplies the lateral portions of the frontal and parietal lobes, as well as the anterior and lateral portions of the temporal lobe, and give rise to perforating branches to the globuspallidus, putamen and internal capsule. The posterior cerebral arteries arise from basilar artery and carry posterior circulation. The posterior cerebral artery (PCA) gives rise to perforating branches that supply thalamic and brain stem and the cortical branches of the posterior and medial temporal lobes and occipital lobes. The cerebral hemispheres are supplied inferiorly by the posterior inferior cerebellar artery (PICA) arising from vertebral artery and superiorly by the super cerebellar artery and anterior laterally by anterior inferior cerebellar artery (AICA) from the basilar artery (Salvador, 2011).

2.1.4 Aetiology and Classification of Stroke

A pathophysiological classification of stroke classified stroke into two groups that is ischaemic stroke and haemorrhagic stroke (Hugh, 2003). Each category can be divided into subtypes that have somewhat different causes and clinical features outcomes and treatment strategies (Louis, 2011). Ischaemic stroke refers to stroke caused by thrombosis, embolism and or systemic hypoperfusion and is more common than haemorrhagic stroke (Salvador, 2011, Louis *et al*, 2011). Haemorrhagic stroke is characterized by too much of blood within the closed cranial cavity (Louis *et al*, 2011). They are more fatal than ischaemic stroke. It is as a result of the extravasations of blood within the brain due to its localization, it can be primarily cerebral or with extension into ventricles, or primary subarachnoid haemorrhage (Guiaslalud, 2011). Intracerebral orintraparenchymatous involves bleeding directly into brain tissue while subarachnoid involves bleeding into the cerebrospinal fluid that surrounds the brain and spinal cord (Louis 2011). Subarachnoid haemorrage results from rupture of most intracranial aneurysms. A subtype non-aneurismal perimesencephalic subarachnoid haemorrhage is also seen to arise from capillary or venous rupture. It is less severe with better prognosis (David, 2011).

2.1.5 **Risk Factors for Stroke**

Risk Factors for stroke could be modifiable or non-modifiable

Non-modifiable risk factors include:

Age is the single most important risk factor of stroke (Ralph *et al*, 1997). Gender increases stroke incidence rate is 1.25 times greater in men, but because women tend to live longer than the men, more women than men die of stroke each year (Ralph *et al*, 1997). Family history; an increase incidence of stroke in families has long been noted (Welin*et al*, 1987. Sickle cell disease is also associated with stroke (Kiely*et al*, 1993). Race increases stroke incidence and mortality varies between racial groups (Ralph *et al*, 1997). Ethnicity: Stroke was a leading cause of death among Native Americans in 1990 (Gillum,1995). Hereditary; There was high incidence of stroke in peoples whose parents have had stroke in the past (Ralph *et al*, 1997).

Modifiable risk factors of stroke include:

Hypertension is the single most important modifiable risk factor for stroke (Ralph *et al*, 1997).

Cardiac Disease various cardiac diseases have been shown to increase the risk of stroke (Ralph *et al*, 1997)

Diabetes mellitus and Glucose metabolism Persons with diabetes mellitus have an increased susceptibility to atherosclerosis and an increased prevalence of atherogenic risk factors, notably hypertension, obesity and abnormal blood lipids (Ralph *et al*, 1997). In persons with diabetes relative risk of ischaemic stroke was 1.8-3.0 (Ralph *et al*, 1997)

Lipids Hypercholesterolaemia is an important risk factor of coronary heart disease, but its link to ischaemic stroke remains uncertain (NCEP, 1993).

Cigarette smoking Increases the risk of ischaemic stroke nearly by up to two times (Shinton and Beevers, 1989).

Alcohol Moderate consumption of alcohol may reduce cardiovascular disease including stroke (Ralph *et al,* 1997).

Illicit Drug Use Drug abuse is a major social problem, cocaine is the substance most commonly associated with stroke (Kelly *et al,* 1992).

Life style factors Various life styles factors have been associated with increased stroke risk (Ralph *et al,* 1997). They include obesity, physical inactivity, diet and acute triggers such as emotional stress (Ralph *et al,* 1997).

Oral contraceptives Oral contraceptives with an oestrogen combination of $\geq$50ug were strongly associated with risk of stroke (Ralph *et al,* 1997).

MigraineMigrainewith aura has been identified as an independent risk factor for ischaemic stroke in men older than 40 in the physicians study (Ralph *et al,* 1997).

Haemostatic and inflammatory factors Fibrinogen has been linked to increased cardiovascular disease risk (Ralph *et al,* 1997).

Homocysteine Blood level of homocysteine produced from essential amino acid methionine can be determined by genetic factors and by intake of B_6, B_{12} and folic acid. Numerous case control studies have shown strong relation between stroke and both basal and post methionine load moderate hyperhomocysteinaemia (Ralph *et al,* 1997).

Vitamin B_{12}Low vitamin B_{12} levels rise sharply along with your risk of stroke.

ZincLow zinc represents an independent risk factor for stroke and therefore a possible target for prevention. (Munshi*et al,* 2010).

Others include anti coagulation, thrombolytic therapy and polymorphism in apolipoprotein E gene (APOE). Some newer risk factors for stroke in general includes cerebral autosomal dominant arteropathy with subcortical infarcts and leucoenchepalopathy point mutation of A3 243G located at chromosome 19q13, Mitochondrial encephalomyopathywith lactic acidosis, dementia, chronic lactic acidosis and episodic vomiting (Ralph *et al*, 1997)

2.1.6 Pathophysiology of Stroke

The three main mechanisms leading to ischaemic stroke are:

1. Thrombosis
2. Embolism
3. Global ischaemia

Atherosclerosis is the most common pathological feature of vascular obstruction resulting in thrombotic stroke. Atherosclerotic plaques can undergo pathological change such as ulcerations, thrombosis, calcification and intra-plaque haemorrhage. The susceptibility of the plaque to disrupt, fracture or ulcerate depends on the structure and composition of the plaque. This process activates main destructive vasoactive enzymes, platelet adherence and aggregation to the vascular wall flow, forming small nod of platelet and fibrin. Leucocytes that are present at the site within 1 hour of the ictus mediate an inflammatory response (Shah, 2013). The effect of ischaemia is rapid because the brain does not store glucose, the chief energy substrate and is incapable of an anaerobic metabolism. Non-traumatic intra cerebral hemorrhage originates from deep penetrating vessels and cause injury to brain tissue by disrupting connection pathways and causing localised pressure injury (Shah, 2013). Acute occlusion of an intracranial vessel causes reduction of blood flow to the brain region it supplies. The magnitude of flow reduction is a function of collateral blood flow and this depends on individual vascular anatomy and the size of occlusion (Wade *et al*, *2005*). In addition to atherosclerosis other pathological conditions that cause thrombotic occlusion of a vessel include clot formation due to hypercoagulable state, fibromuscular dysplasia, giant cell arteritis and dissection of vessel wall.

2.1.6.1 Molecular Pathophysiology of Stroke

At molecular level of development, the hypoxic-ischaemic neuronal injury is greatly influenced by “over action” of certain neurotransmitters, primarily glutamate and aspartate. This process is called “excitotoxicity” and is triggered by depletion of cellular energy stores. Glutamate, which is normally stored inside the synaptic terminals, is cleared from the extracellular space by an energy dependent process. The greatly increased concentration of glutamate and aspartate in extracellular space in a depleted energy state result in the opening of calcium channels associated with N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-Isoxanole propionate (AMPA) receptors. Persistent membrane depolarisation causes influx of calcium, sodium and chloride ions and efflux of potassium ions (Shah, 2013).

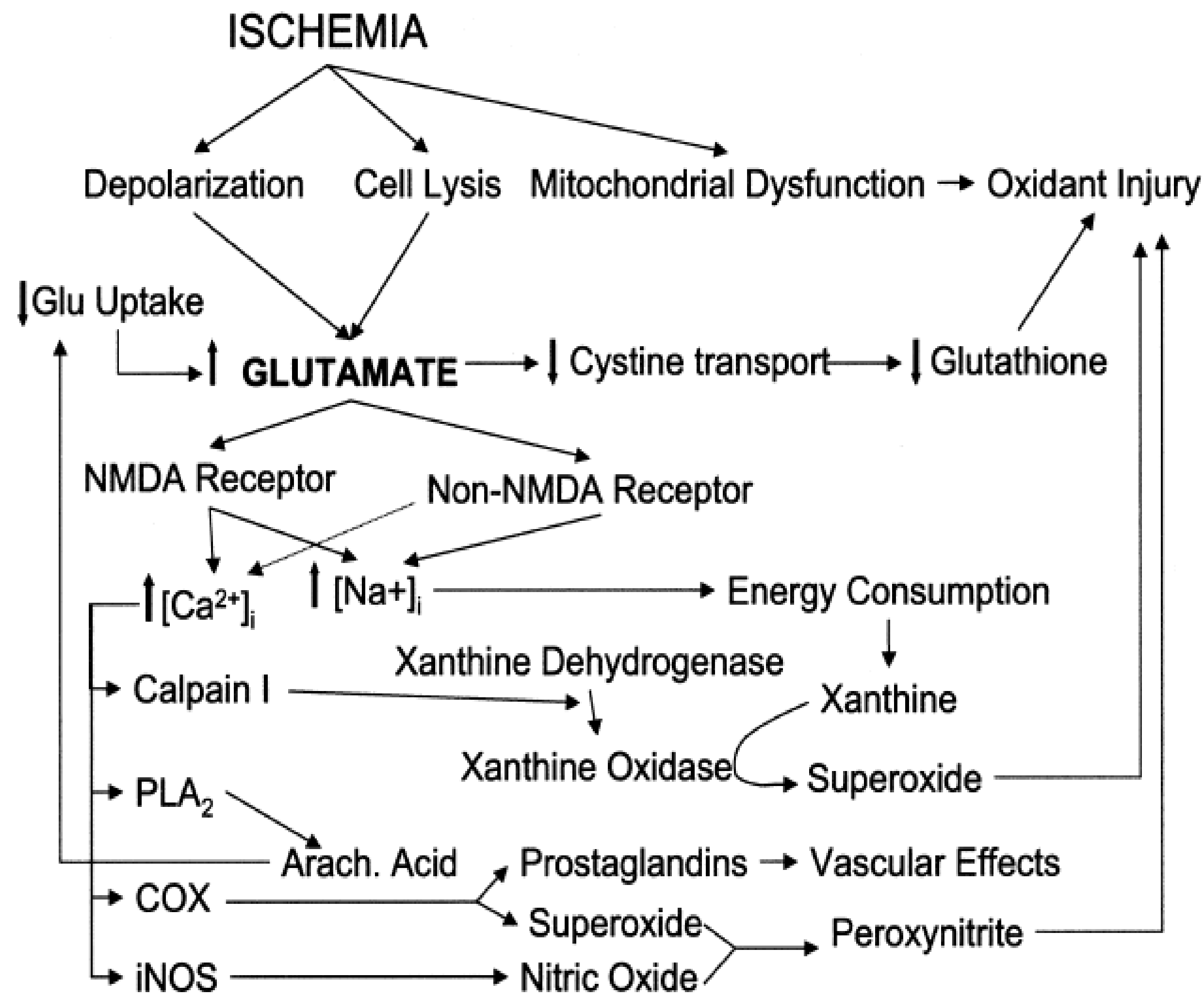

Fig 2.1 Schematic diagram illustrating the mechanism by which ischaemia and excito-toxicity injure neurons is shown.

2.1.6.2 Pathology of Stroke

Macroscopically ischaemic stroke features changes with time. During the first 6 hours of irreversible injury little can be observed by 48 hours, the tissue becomes pale, soft and swollen, and the brain corticomedullary junction becomes distinct from 2 to 10 days. From 10 days to 3 weeks the tissue liquefies, eventually leaving a fluid filled cavity lined by dark grey tissue, which gradually expands as dead tissue is removed (Cotran *et al, 2003*).

Microscopically, the early lesions show a central core of clotted blood surrounded by a rim of brain tissue showing anoxic neuronal and glial changes as well as oedema. Eventually oedema resolves, pigment and lipid laden macrophages appear and proliferation of reactive astrocytes is seen at the periphery of the lesion. In a hypertensive stroke macroscopically the brain is swollen the gyri are widened and sulci narrowed, the cut surface shows poor demarcation between grey and white matter. In the first 12-24hours red neurons with microvacuolisation and eosinophilia of neuronal cytoplasm are seen, later pyknosis and karyorrhexis are seen between 24 hours and 2wks. If there is necrosis of tissue, influx of macrophages, vascular proliferation and reactive gliosis occur. After 2 weeks there is removal of all necrotic tissue and loss of normally organized CNS structure and gliosis (Cotran *et al, 2003*).

2.1.7 Clinical Features

Following a stroke the resulting functional restriction can vary greatly. The effect of stroke depends on the part of brain affected and the extent of damage. Functional restrictions resulting from stroke include;

Paralysis, cognitive deficits, personality changes, visual disturbances, speech and language problems, fatigue, emotional difficulties.Pain might also result following stroke, a severe sudden headache without apparent cause, sudden numbness, weakness or dizziness (UCSF, 2012). If a stroke involves pons it leads to hyperpyrexia and deep coma.

2.1.8 Investigation of Patient with Stroke

Investigations are done to achieve the following;

1. To confirm diagnosis if in doubt
2. To establish any treatable cause
3. To establish baseline so as to assess if patient is improving or deteriorating
4. To determine any risk factor which might be treatable to prevent recurrence
5. To predict likelihood of immediate complications
6. To exclude other possibilities

Investigations done to a stroke patient includes: Haematological, radiological, biochemical and microbiological.

2.1.8.1 Haematological

Haematological includes, full blood count protein C and S assay, Haemoglobin genotype, Erythrocyte sedimentation rate etc

2.1.8.2 Radiological

This include; Computerized tomography scan and magnetic resonance imaging, digital subtract angiography, doppler ultrasound, computerized tomography angiography and magnetic resonance angiography, othertranscranial Doppler, echocardiography, electrocardiogram, chest X ray etc.

2.1.8.2 Microbiological/Immunological

These tests include; Venereal disease research laboratory test (VDRL), human immunodeficiency virus screening.

2.1.8.4 Biochemical

Serum glucose concentration, lipid profile, urea and electrolytes, urinalysis, serum homocysteine, serum vitaminB_{12},zincetc are important investigations in the assessment of patients admitted with stroke.

2.2 SOME BIO MARKERS OF STROKE

2.2.1 Homocysteine

Is a protein amino acid and a homologue of the amino acid cysteine, differing by an additional methylene bridge (-CH_2-). It is biosynthesized from methionine by the removal of its terminal C^{ε} methyl group. Homocysteine can be recycled into methionine or converted into cysteine with the aid of B-vitamins. Homocysteine is not obtained from the diet; it is biosynthesized from methionine via a multi-step process. First, methionine receives an adenosine group from ATP, a reaction catalyzed by S-adenosyl-methionine synthetase, to give *S*-adenosyl methionine (SAM). SAM then transfers the methyl group to an acceptor molecule, (i.e., norepinephrine as an acceptor during epinephrine synthesis, DNA methyltransferase as an intermediate acceptor in the process of DNA methylation). The adenosine is then hydrolyzed to yield L-homocysteine. L-homocysteine has two primary fates: conversion via tetrahydrofolate (THF) back into L-methionine or conversion to L-cysteine.

2.2.2 Vitamin B_{12}

Vitamin B_{12}, also called cobalamin, is a water-soluble vitamin with a key role in the normal functioning of the brain and nervous system, and for the formation of blood. It is one of the eight B vitamins.

2.2.3 Zinc

Zinc is a metallic chemical element; it has the symbol Zn and atomic number 30. Zinc is the 24th most abundant element in the earth's crust and has five stable isotopes. Zinc deficiency affects about two billion people in the developing world and is associated with many diseases. Enzymes with a zinc atom in the reactive center are widespread in biochemistry, such as alcohol dehydrogenase in humans (Wikipedia, 2014).

2.2.4 Homocysteine in Acute Ischaemic Stroke

Increased homocysteine causes premature atherosclerosis and accelerated vascular disease (Pitchaiah, 2013). Mechanism of thromboembolism is multiple and could be due to, endovascular dysfunction and endothelial cell damage due to deficient nitrous oxide and oxidative stress, lipid peroxidation, smooth muscle cell proliferation and hypercoagulability due to increased thrombosis, upregulation of prothrombotic factors (XII and V) and platelet activation. This may affect stability of arterial wall and cause dissections, arterial thrombosis, and arteriopathy (Testai and Gorelick, 2010)

Studies show, however, that elevated homocysteine precedes the onset of stroke. This strongly indicates that homocysteine, rather than just being a marker for cardiovascular disease, actively contributes to the disease process (Strokes, 2014)

2.2.5 Vitamin B_{12} in Acute Ischaemic Stroke

Vitamin B_{12} deficiency lurks silently, gradually increasing its victim's risk of stroke, because B_{12} allows folic acid, to convert homocysteine into methionine. When B_{12} levels drop to an unhealthy point, this process breaks down and homocysteine levels rise sharply along with risk of heart attack or stroke (Strokes, 2014)

Vitamin B_{12} deficiency is not always easy to detect. A study published by Eric et al using a highly accurate screening method called the urinary methylmalonic acid (uMMA) test identified undiagnosed B_{12} deficiency in the study's participants , this undiagnosed group was 2.6 times as likely to suffer a stroke(Strokes, 2014).

2.2.6 Zinc in Acute Ischaemic Stroke

Nearly two decades ago, zinc was first implicated in the pathogenesis of ischemia and more than twenty additional in vivo studies have since examined the role of zinc during both global and focal experimental paradigms. From these studies, zinc has been reported to possess both neurotoxic and neuroprotective capabilities during experimentally-induced ischemia (Sherri and Richard, 2007).

Substantial evidence supports the hypothesis that neural cell death occurring for days or weeks after the initial blockage or hemorrhage is the result of an excitotoxic cascade involving metal ions, especially calcium. Evidence is reviewed here that zinc is a potent toxin released from cellular sites during and after ischemia that can cripple the cell's energy production and ability to defend itself from the well-known effects of oxidative stress. In particular, the protonation of histidinyl zinc ligands due to lactic acidosis and the oxidation of thiol ligands both can release bound intracellular zinc irrespective of any channel opening, and zinc has been shown to be a potent inhibitor of mitochondrial enzymes involved in energy production and the destruction of reactive oxygen species. There is disagreement as to whether elevated zinc ion levels induce apoptosis. These results suggest that zinc ion may play a substantial role in the toxic events following stroke, and may suggest alternative therapies (Richard, 2012)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 BACKGROUND OF STUDY AREA

This was a cross-sectional descriptive study, which was conducted at Ahmadu Bello University Teaching Hospital Shika, Zaria. The predominant occupation of the people of Zaria is farming but civil servants and businessmen also form a sizeable part of the population. The predominant religions are Islam and Christianity. Zaria has a tropical climate with a temperature range from 15.3-36.2^{0}C and a rainfall that ranges from 0 – 816mm/month. Zaria has two main seasons, the rainy season from April to October and dry season from November to March.

3.2 STUDY POPULATION

3.2.1 Subjects

A total of 200 subjects were recruited for the study. These were made up of ischaemic stroke patients admitted to the Accident and Emergency unit and medical ward of ABUTH Shika Zaria and another 100 apparently healthy ages and sex-matched controls. The controls group were recruited from Samaru, Zaria city, Milgoma and Shika communities after they gave an informed consent to participate in the study. Serum homocysteine, vitamin B_{12} and zinc were measured in the one hundred acute ischaemic stroke patients which was confirmed by brain CT-Scan or Siri-raj stroke score of less than minus one (-1)and the control group.

3.2.2 Inclusion Criteria for Patients and controls

All prospective patients newly admitted within 72 hours with diagnosis of acute ischaemic stroke that were confirmed by CT- Scan or with Siri raj stroke score below -1 who consented or whose patients care gave informed consent for the study. The controls were all apparently healthy individuals who were aged and sex matched with patients with no previous history of stroke and renal failure who were not on methotrexate, tamoxifen, L- DOPA, phenytoin , bile acid sequestrants, Vitamin B12, folic acid use of oral contraceptive pills, anticonvulsants or lipid lowering drugs and zinc supplements that consented to the study.

3.2.3 Exclusion Criteria for Patients and controls

Patients who are on medication that would affect homocysteine zinc and vitamin B_{12} levels such as methotrexate, tamoxifen, L- DOPA, phenytoin , bile acid sequestrants, Vitamin B_{12}, folic acid , use of oral contraceptive pills, anticonvulsants or lipid lowering drugs and zinc supplements and or with renal insufficiency, liver disease, thyroid disease, leukaemia or psoriasis.Patients with ischaemic stroke or patients' care giver who refused to give informed consent for the study. It also included all apparently healthy individuals with same ageand sex as that of the patient who did not consent to the study.

3.2.6 Informed Consent

Informed written consent was obtained from the controls, the patients, or their care givers using approved protocol (appendices I and II).

3.2.7 Sample Size Determination

The sample size was determined using the Fisher's formula (Singha, 1996**)**

$$N = \frac{Z^2 pq}{d^2}$$

Where:

N= Sample size

P- Prevalence of problem (over all prevalence of stroke usually set at 5% or 0.05) (Bots et al, 1996).

q= 1-p

Z= 95% confidence interval = 1.96

$$N = (\frac{1.96)^2 x\ 5\%\ x\ (1 - 0.05)}{(0.05)^2}$$

$$= \frac{3.8\ x\ 0.05\ x\ 0.95}{0.0025}$$

$$n = 73$$

The number was rounded off by 17% to give a final sample of size of 100 stroke patients because of possibility of dropouts(Anjanette*et al*, 2005)

3.2.8 Ethical Approval

Approval was obtained from Ethical and Scientific committee of ABUTH, Zaria before embarking on the study. The investigation was at no cost to the patients.

3.3 STUDY PROTOCOL

A questionnaire (appendix III) attached to this study was administered to all subjects. It contained biodata, risk factor profile and co-morbid conditions. The data collected from the patients and the control included age, sex, weight, blood pressure and/or history of hypertension, diabetes mellitus, smoking, alcohol consumption, previous thrombotic episodes, and history of oral contraceptive use in women. Brain CT-Scan was used for stroke confirmation and where this was not available Siri-raj stroke score was used, as is the case in most studies on stroke in sub-Saharan Africa(Myles, 2007). The patients did not bear the cost of any of the investigations.

The following biochemical investigations were performed on all study subjects; serum homocysteine, vitamin B_{12} and zinc. Stroke severity was determined using National Institute of Health Stroke Score (NIHSS)

The NIHSS is a 15-item impairment scale which provides a quantitative measure of key components of standard neurological examination (Appelros*et al* ,2004, Brott*et al,* 1989) The scale assesses level of consciousness, extraocular movement, visual fields, facial muscle function, extremity strength, sensory function, coordination (ataxia), language (aphasia), speech (dysarthria) and hemineglect (Lyden*et al,* 1999, Lyden*et al*, 2001) The scale was developed for use in acute stroke therapy trials and has since been widely used as standard part of assessment in clinical trials (Brett *et a,l* 2002) . A high score correlates with large infarction and strongly predicts the likelihood of a patient morbidity after stroke. Stroke severity based on NIHSS Scoring system, was classified as mild, moderate or severe (Janet *et al*, 2001). Patients with NIHSS score of 1-4 was regarded as mild stroke 5-15 were regarded as moderate stroke and 16 - 42 classified as severe stroke.

3.3.1 Siri Raj Stroke Score

(a) Level of consciousness at onset

0= Alert 1= Drowsy/stupor

2=Coma

(b) Vomiting 0= Absent 1= Present

(c) Headache 0=Absent 1= Present

(d)Atheroma markers (History of DM, Angina, Intermittent claudication)

0= Absent 1=Present

SRScore (2.5×(a)__)+(2×(b)__)+(2×(c)__)+(0.1×DBP___)−(3×(d)__)−12

TOTAL=

Summary: ☐ Ischaemic stroke (score below −1)

☐ Haemorrhagic stroke (score above 1)

☐ Equivocal (score between 1and -1)

3.4 SPECIMEN COLLECTIONS AND PROCESSING

The antecubital fossa was cleaned with methylated spirit and allowed to dry. Five milliliter of fasting blood samples were obtained from the forearm of each subject using a 5ml syringe and a 21g needle from anterior cubital vein. The blood was transferred into a plain bottle and allowed to stand for about 30 minutes for it to clot and retract. This was then centrifuged for 5 minutes at 10000rpm.The serum was separated from the cells and transferred into plain (sample) bottles and then frozen at -80^{0}C in deep freezers until the time for analysis that was when the last sample was collected in April 2014. Samples of patients were collected simultaneously with that of healthy controls. Samples were analyzed in batches of 96.

3.5 CHEMICALS

The chemicals and kits used for measurements of serum homocysteine, vitamin B_{12} and zinc were procured from Wkea medical supplies corporation, changchum china, diagonostic automation/ cortezdiagonostics USA, and labkit, chemlexs.a. Barcelona Spain respectively.

3.6 EQUIPMENT

- Hettich Universal 32 centrifuge (Germany) was used to spin the blood specimen. Manufactured by DJB Labcare Ltd 20 Howard Way Interchange Business Park Newport Pagnell Buckinghamshire MK16 9QS England
- Beckman Coulter DU-20 general purpose Ultraviolent/Visible spectrophotometer was used for measuring absorbance of zinc**.Manufactured by** Beckman Coulter South Africa Pty Ltd
- Bio Rad PR-5100, Vamed Engineering Nigeria Limited, L10000-ZR-002, Micro plate reader.

3.7 ANALYTICAL METHODS

3.7.1 Measurement of Serum Homocysteine (Frantzen et al 1998)

Serum homocysteine concentrations were measured by Enzyme Linked Immunosorbent assay (ELISA)

3.7.1.1 Principle

This is by using purified human homocysteine to coat microtitre plate well, and making a solid phase antibody, then homocysteine was added to the wells. Combined homocysteine antibody with enzyme label, become antibody antigen complex, which was then washed after washing completely, substrate was added, substrate became blue in colour at HRP enzyme catalyzed, reaction was terminated by the addition of a sulphuric acid solution and the colour change was measured spectrophotometrically at a wave length of 450nm.The concentration of homocysteine in the samples is then determined by comparing the Optical Density of the samples to the standard curve.

3.7.1.2 Procedure

Calibrator, control and patient samples were simultaneously incubated with the enzyme labeled antibody. At the end of the assay incubation, the microwell was washed to remove unbound components. The substrate tetramethylbenzidine (TMB) an acidic solution was added to stop the reaction. The optical density was determined within 15mins by a microplate reader at 450nm.

3.7.1.3 Calculation

The intensity of the yellow color is directly proportional to the concentration of homocysteine in the sample. A dose response curve of absorbance unit vs. concentration is generated using results obtained from the calibrators. Concentrations of homocysteine present in the control's and patient's samples are determined directly from this curve.

3.7.2 Measurement of Serum Vitamin B_{12}(Perlmann and Engvall 1971)

Serum vitamin B_{12} concentrations were measured by Enzyme Linked Immunosorbent assay (ELISA).

3.7.2.1 Principle

This test was based on delayed competitive enzyme immunoassay, the essential reagents required for an enzyme immunoassay include antibody, enzyme- antigen conjugate and native antigen.

3.7.2.2 Procedure

Biotinylated antibody was mixed with a serum containing the antigen, a reaction occurred between the antigen and the antibody, after a short incubation the enzyme conjugate was added, and competition reaction resulted between the enzyme analog and the antigen in the sample for a limited number of antibody binging sites. A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurred which led to separation of the antibody bound fraction after aspiration.

3.7.2.3 Calculation

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve was generated from which the antigen concentration of an unknown was ascertained.

3.7.3 Measurement of serum Zinc(Johansen and Eliassonn, 1987).(Fouda*et al,* 2014)

Serum Zinc was measured using direct colorimetric NITRO-PAPS method

3.7.3.1 Principle

Direct colorimetric test without deproteinazation of the sample was used at pH 8.2 in a buffered media. Zinc reacts with the specific complexant NITRO-PAPS, and forms a stable coloured complex. Zn forms a red chelate complex with 2-(5-bromo-pyridylazo)-5-(npropyl-*N*-sulphopropylamino)-phenol. The absorbance is measured at 560 nm and is proportional to the concentration of total zinc in the sample. The colour intensity is proportional to the amount of zinc present in the sample.

3.7.3.2 Procedure

Test tubes were prepared and labeled as blank test and standard. 0.8mls of R1 reagent containing 0.4mol/l of Borate buffer, and 0.2mls of R2 reagent containing 0.4mmol/l of NITRO-PAPS colour was added to each tube. Fifty microlitre of serum was added to test,fifty microlitre of standard and fifty microlitreof distilled water to blank. The test tubes were mixed thoroughly and incubated for 5 minutes. The coloured solution was read at 578nm, the colour was stable for up to 30 minutes. The blank was used to set the spectrophotometerat zero.

3.7.3.3 Calculation

Serum zinc (µg/dl) = $\frac{Atest}{Astandard} x Cstandard$

A= absorbanceC=concentration

3.8 QUALITY CONTROL

Quality Control material was obtained from pooled sera for homocysteine and vitamin B_{12}. Commercially prepared quality control materials (normal and pathological) bought from labkitchemlexs .a pol. ind.spain were used for Zinc. All assays were done in duplicates. Coefficient of variation was calculated from mean and standard deviations obtained.

3.9 STATISTICAL ANALYSIS OF THE RESULTS

Statistical Package for Social Sciences 16.0 (SPSS 16.0) for windows (SPSS Inc. Chicago 16) was used for statistical analysis of the results. Serum homocysteine, vitamin B_{12} and zinc of ischaemic stroke patients were compared with those of the controls using independent t-test (two tailed). Categorical data was summarized as frequencies and percentages while continues data were summarized as mean and standard deviation. Discrete variables were analyzed using chi square. Students-t-test were used to analyze continues normally distributed variables. Less than 0.05 was considered significant statistically.

CHAPTER FOUR

4.0 RESULTS

4.1 CLINICAL AND DEMOGRAPHIC CHARACTERISTICS OF STUDY POPULATION

This prospective cross sectional descriptive study was carried out in ABUTH over a period of thirteen months; from March 2013 to March 2014, during which 246 patients were admitted with diagnosis of acute stroke, but 146 patients were excluded for various reasons. Biodata for both controls and stroke patients are shown in table 4.1. One hundred patients with diagnosis of acute ischaemic stroke were recruited as the study groups (cases) while the same number of age and sex matched were recruited as controls (healthy subjects). The mean± SD of the patients' age was 59±14.08 years. It showed predominance of males (62%) over female (38%) with a male to female ratio of 1.6: 1 in both patients (cases) and control groups. The majority of the subjects were Hausa /Fulani which accounted for 74%and 54% for cases and controls respectively. Systemic hypertension was found to be the most common modifiable risk factor for cerebrovascular disease accounting for 91% of cases followed by diabetes mellitus which accounted for 8%, and sickle cell anaemia 1%. The mean (± SD) systolic blood pressure for cases was 156 (±32) mmHg which was significantly higher ($p < 0.0001$) than that of controls which was 120(20); mean (± SD) diastolic blood pressure of cases was 94 (20) mmHg which was significantly higher ($p < 0.0001$) statistically than that of controls which was 76 (7) mmHg.

4.2 ADMISSION HOMOCYSTEINE, VITAMIN B_{12} AND ZINC (MEAN±SD) IN STROKE CASES AND CONTROLS

Pattern of serum homocysteine, vitamin $B_{12}$and zinc of stroke acute ischaemic patients and controls were shown in table 4.2. Acute ischaemic stroke patients had higher serum homocysteine, and lower vitamin B_{12} and zinc than corresponding healthy controls and the difference was found to be statistically significant ($p<0.05$), with an odds ratio of 0.04, 0.19 and 0.54 for serum homocysteine, serum vitamin $B_{12}$and serum zinc respectively.

4.3 REFERENCE INTERVALS OF SERUM HOMOCYSTEINE,VITAMIN B_{12} AND ZINC USING HEALTHY CONTROLS.

The reference interval of serum homocysteine, vitamin B_{12} and zinc in the apparently healthy control population, was determined using the mean value ± 2 standard deviation of the controls, and was found to be 0.9 - 1.70 µmol/l , 199.72 - 685.48 pg/ml and 52.26 - 111.86 µg/dl for homocysteine, vitamin B_{12} and zinc respectively as shown in table 4.3.

4.4 SERUM LEVELS OF HOMOCYSTEINE VITAMIN B_{12} AND ZINC OF ISCHAEMIC STROKE PATIENTS BASED ON DIFFERENT MODIFYING RISK FACTORS.

There was no difference in serum concentrations of homocysteine, vitamin B_{12} and zinc between hypertensive ischaemic stroke patients and normotensive ischaemic stroke patients p =0.057, 0.179 and p= 0.120 respectively as shown in table 4.4. Alsothere was no difference in serum concentrations of vitamin B_{12} and zinc between diabetic ischaemic stroke and that of non-diabetic ischaemic stroke patients p =0.227 and p= 0.093 respectively, however the serum homocysteine of diabetic patients was significantly higher than that of non-diabetic patients p=0.024 as shown in table 4.5. Table 4.6 showed that there was no difference in serum concentrations of homocysteine vitamin B_{12} and zinc between the non-elderly (age less than 65 years) ischaemic stroke and that of elderly people (age 65years and above) ischaemic stroke patient (p =0.467, 0.318 and p= 0.067) respectively. There was also no difference in serum concentrations of homocysteine vitamin B_{12} and zinc between the male ischaemic stroke and female ischaemic stroke patients (p =0.959, 0.126 and p= 0.452) respectively as shown in table 4.7.

Table 4.1: Clinical and demographic characteristics of study population

Demographic characters	Acute Ischaemic stroke patients (n=100)	Control(n=100)	P value
AGE			
• mean(range)	59(19-96)	59(19-96)	
• < 65(freq)	75	75	
• ≥65(freq)	25	25	
SEX			
• Male	62	62	
• Female	38	38	
ETHNICITY			
• Hausa/ Fulani	74	54	
• Others	26	46	
RISK FACTOR IN PATIENTS			
• Hypertension	91	0	
• Diabetes mellitus	8	0	
• Sickle cell anaemia	1	0	
BLOOD PRESSURE (mmHg)			
• systolic	156(32)	120(12)	<0.0001*
• diastolic	94(20)	76(7)	<0.0001*

Table 4.2 Serum homocysteine, vitamin B_{12} and zinc (mean (±SD) in stroke patients and controls

Analytes	Patients (n=100)	Control (n=100)	Odds ratio	p-values
Homocysteine(µmol/L)				
• Mean(±SD)	1.62(0.35)	1.30(0.20)	0.04	<0.0001*
Vitamin B_{12}(pg/ml)				
• Mean(±SD)	137.15(100.92)	442.60(121.44)	0.19	<0.0001*
Zinc(µg/dL)				
• Mean(±SD)	61.44(25.1)	82.06(14.90)	0.51	<0.0001*

n=frequency, SD=standard deviation Statistically significant ($p<0.05$)

Table 4.3 Reference intervals of serum homocysteine, vitamin B_{12} and zinc using healthy controls.

Analytes	Mean	SD	Reference interval (mean ± 2SD)
Homocysteine(µmol/L)			
• (mean±2SD)	1.30	0.20	0.90 -1.70
Vitamin B_{12}(pg/ml)			
• (mean±2SD)	442.60	121.44	199.72- 685.48
Zinc(µg/dL)			
• (mean±2SD)	82.06	14.90	52.26- 111.86

SD= Standard deviation hcy=homocystieine, vit B_{12} = vitamin B_{12}

Table 4.4 Serum levels of homocysteine vitamin B_{12} and zinc in hypertensive and normotensive ischaemic stroke patients.

Analytes	Hypertensive (n=91)	Normotensive (n=9)	p-values
Homocysteine(µmol/L)			
• Mean(±SD)	1.60(0.349)	1.83(0.25)	0.057
Vitamin B_{12}(pg/ml)			
• Mean(±SD)	141.43(103.53)	93.88(56.05)	0.179
Zinc(µg/dL)			
• Mean(±SD)	62.67(24.99)	49.00(24.05)	0.120

n = frequency, SD = Standard deviations, Statistically significant if $p < 0.05$

Table 4.5 Serum levels of homocysteine vitamin B_{12} and zinc of ischaemic stroke patients with and withoutdiabetes.

Analytes	Diabetic (n=9)	Non- diabetic (n=91)	p-values
Homocysteine(µmol/L)			
• Mean(±SD)	1.88(0.20)	1.60(0.348)	0.024*
Vitamin B_{12}(pg/ml)			
• Mean(±SD)	95.62(59.66)	140.76(103.16)	0.227
Zinc(µg/dL)			
• Mean(±SD)	47.12(24.99)	62.68(24.86)	0.093

n = frequency, SD = Standard deviations, Statistically significant if $p< 0.05$

Table 4.6: Serum levels of serum homocysteine vitamin B_{12} and zinc of elderly and non-elderly ischaemic stroke patients

Analytes	Non-elderly (≤ 65yrs) (n=75)	Elderly (>65yrs) (n=25)	p-values
Homocysteine(µmol/L)			
• Mean(±SD)	1.60(0.36)	1.66(0.29)	0.467
Vitamin B_{12}(pg/ml)			
• Mean(±SD)	143.00(109.98)	119.60(65.47)	0.318
Zinc(µg/dL)			
• Mean(±SD)	58.78(22.98)	69.40(29.72)	0.067

n = frequency, SD = Standard deviations, Statistically significant if $p < 0.05$

Table 4.7; Serum levels of serum homocysteine, vitamin B_{12} and zinc of male and female ischaemic stroke patients.

Analytes	Male (n=62)	Female (n=38)	p-values
Homocysteine(µmol/L)			
• Mean(±SD)	1.62(0.36)	1.62(0.31)	0.959
Vitamin B_{12}(pg/ml)			
• Mean(±SD)	149.27(110.59)	117.37(80.24)	0.126
Zinc(µg/dL)			
• Mean(±SD)	59.95(23.16)	63.86(28.14)	0.452

n = frequency, SD = Standard deviations, Statistically significant if $p < 0.05$

4.5 FREQUENCY OF ELEVATED HOMOCYSTEINE, LOW VITAMIN B_{12} AND LOW ZINC AMONGHEALTHY CONTROLS AND PATIENTS WITH ISCHAEMIC STROKE

Table 4.8 shows 34 acute ischaemic stroke patients (34%) of cases had high and 66 patients (66%) had normal serum homocysteine. Only two of the controls (2%) had high and the remaining 98 controls (98%) had normal serum homocysteine. The difference was found to be statistically significant ($p<0.05$), while table 4.9 showed 81 patients (81%) of had low and 19 patients(19%) had normalserum vitamin B_{12} , while all the control had normal vitamin $B_{12}$and the difference was found to be statistically significant ($p<0.05$). Forty six patients (46%)of acute ischaemic stroke patients had low and 54 patients (54%) had normalzincas shown in table 4.10, while all the control had normalzincand the difference was found to be statistically significant ($p<0.05$).

4.6 SERUM LEVEL OFHOMOCYSTEINE VITAMIN B_{12} AND ZINC OF STROKE PATIENTS BASED ON SEVERITY

According to NIHSS score stroke scale 11acute ischaemic stroke patients (11%) were found to have score of 1-4 which is (mild) stroke, 74 patients (74%) had score of 5-15 which is (moderate) stroke, while 15 patients (15%) had 16-42 which is (severe) stroke. Vitamin B_{12} and zinc were lower in severe stroke with higher homocysteine levels but no significant difference was observed statistically between the three different groups of stroke severity ($p = 0.548$, 0.301 and $p=0.626$) respectively as shown in table 4.11.

4.7RELATIONSHIP BETWEEN STROKE SEVERITY AND ANALYTES ABNORMALITIES.

Stroke patients with higher homocysteine, and lower vitamin B_{12} and zinc concentrations had more severe neurological deficit than patients with normal values of homocysteine, vitaminB_{12} and zinceven though the difference was not statistically significant with p values of 0.946, 0.735, and 0.566 respectively as shown in table 4.12. Their corresponding NIHSS score mean(±SD) were 10.96 (5.23),10.89 (4.73)and 11.11(5.23) forstroke patients with higher homocysteine, and lower vitamin B_{12} and zinc concentrations respectively, while that of patients with normal homocysteine vitamin B_{12} and zinc values had NIHSS score mean (±SD) of 10.83(4.55),10.47(5.05) and10.56 (4.36) respectively.

4.8 CORRELATION BETWEEN SERUM HOMOCYSTEINE AND VITAMIN B_{12} AMONG STROKE PATIENTS

There was significant negative correlation between serum homocysteine and vitamin B_{12} among cases with p-value of 0.04 and r-value of – 0.198 as shown in table 4.13.

Table 4.8Frequency of elevated homocysteine among patients with ischaemic stroke and apparently healthy controls

Analyte	Patients(n)%	Controls(n)%
Normal homocysteine(≤1.70µmol/L)	66	98
High homocysteine(>1.7µmol/L)	34	2
Total (n)	100	100

Fishers exact test = 40.601, n = frequency, p value= <0.005 statistically significant

Table 4.9 Frequency of low vitamin B_{12} among patients with ischaemic stroke and apparently healthy controls

Analyte	Patients (n)%	Controls(n)%
Low vitaminB_{12} (<199.72pg/ml)	81	0
Normal vitamin B_{12} (199.72-685.48pg/ml)	19	100
Total (n)	100	100

Fishers exact test = 166.896 n = frequency, p value = <0.05(statistically significant)

Table 4.10 Frequency of low zinc among patients with ischaemic stroke and apparently healthy controls

Analyte	Patients(n)%	Controls(n)%
Low zinc (<52.26µg/dl)	46	0
Normal zinc (52.26-111.86µg/dl)	54	100
Total (n)	100	100

Fishers exact test = 74.873 n = frequency, p value= <0.05(statistically significant)

Table 4.11Admission serum homocysteine, vitamin B_{12} and zinc based on stroke severity

Analytes	NIHSS score			
	Mild n=11	Moderate n=74	Severe n=15	P -value
homocysteine(μmol/L)				
• Mean (±SD)	1.68(0.45)	1.60(0.34)	1.69(0.24)	0.548
vitaminB_{12}(pg/ml)				
• Mean (±SD)	134.09(80.95)	145.00(106.66)	100.66(79.12)	0.301
zinc(μg/dl)				
• Mean (±SD)	59.90(26.54)	62.77(24.08)	56.00(29.76)	0.626

NIHSS= National Institute of Health Stroke Score, SD=standard deviation,n=frequency Statistically significant ($p<0.05$)

NIHSS SCORE:--------------------INTERPRETATION FOR NIHSS Score

1-4 = Minor Stroke
5-15 = Moderate Stroke
16-42 = Severe Stroke

Table 4.12 Relationship between stroke severity and abnormalities of the measured analytes.

Analytes	NIHSS score Mean(±SD)	P value
Normal hcy (µmol/L)	10.83(4.55)	
High hcy (µmol/L)	10.96(5.23)	0.946
Low vitB_{12} (pg/ml)	10.89(4.73)	
Normal vitB_{12} (pg/ml)	10.47(5.05)	0.735
Low zinc(µg/dl)	11.11(5.23)	
Normal zinc (µg/dl)	10.56(4.36)	0.566

NIHSS= National Institute of Health Stroke Score,hcy= homocysteine, vit B_{12}= Vitamin B_{12} SD= Standard deviation

Table 4.13 Correlation between serum homocysteine and vitamin B_{12} among acute ischaemic stroke patients.

Analytes	r-value	p-value
Hcy/vit B_{12}	$- 0.198^{S}$	0.04

hcy= homocysteine , vitB_{12}= vitamin B_{12} r= correlation coefficient S= significance

CHAPTER FIVE

5.0 DISCUSSION

The present study was designed to determine the relationship between serum homocysteine, vitamin B_{12} and zinc and acute ischaemic stroke in Nigerians. Homocysteine, vitamin B_{12} and zinc in ischaemic stroke patients and controls was measured; and thus prevalence levels determined. The relationship between homocysteine, vitamin B_{12} and zinc levels (measured within 72 hours of acute ischaemic stroke onset) in patients with acute ischaemic stroke evaluated.

Despite significant advances in the understanding of its underlying pathophysiology and the development of more effective methods of its management, stroke continues to be a leading cause of mortality and physical disability worldwide (Bonita*et al,* 1984,Bogousslavsky*et al,* 2000,Casper *et al,* 2003,Osuntokun*et al,* 1979,Walker *et al,* 1994). Present study showed a mean age of 59years for the ischaemic stroke patients recruited which was similar to other studies reported (Amu *et al,* 2005) and that of Nura*et al,* 2006 in Maiduguri. In relation to sex male preponderance with a male to female ratio of 1.6:1 was observed in the study, this ratio is slightly higher than that reported from Benin and Lagos(Ralph *et al,* 1997) greater but similar to the report of Sani*et al,* 2013 from Sokoto. Stroke is said to be more common in male than females as confirmed by this study, this may be due to religious or some cultural practices in this part of the country where females do not regularly go for orthodox help this may explain the increase in male preponderance however Ogun*et al,* 2005 reported higher females than males, thus incidence of stroke in suggested is due to changing the of in sub-Saharan Africa.It has been reported that the male sex is a risk factor especially for thrombotic stroke, until the eighth and ninth decades when gender plays no role in enhancing risk (Aghaji*et al,* 1989) but the reason for this is not quite obvious. It may be that the female sex hormones are protective before menopause or that the females tolerate hypertension better than the males. The other possibilities include the influence of cultural factors, like the ready accessibility of males to health care and the greater life expectancy found in women.

This study of acute ischaemic stroke showed predominance of hypertension as a major risk factor, this finding is similar to what was reported by other studies such as that of (Ralph *et al,* 1997 and Nura*et al,* in 2006). Hypertension was found in 91 patients (91%) with diabetes mellitus being in only (8%) of the studied patients.Alahussain*et al*found (47.1%) of his patients to be hypertensive while (18.6%) being diabetic mellitus as risk factors of stroke (AlaHussain*et al,* 2010). Seventy four percent (74%) of our stroke patients were fromHausa/Fulani ethnic group with other ethnic groups 26%. This is not a surprise because majority of the population of the study area belong to that ethnic group. Hypertension and diabetes mellitus and other non-communicable diseases are becoming more prevalent in Africa and sun Saharan Africa in 2002 by Straus et al. Hypertension is the most predominant risk factor for those with first time stroke or those with recurrent stroke(Osuntokun*et al,* 1979,Walker *et al,* 1994,Bwala*et al,* 1989, Nwosu*et al,* 1992). It is not surprising to find a lot of patients being hypertensive factors attributing to the rise were change in life style, sedentary life style, cigarette smoking, etc, Studies have shown that modifying this factors will improve the prevalence and therefore outcome of hypertension and its complication especially stroke **(**Bwala*et al,* 1989, Nwosu*et al,* 1992, Osuntokun*et al,*1977)

Serum levels of homocysteine in the studied patients was significantly higher compared to the controls. This study confirmed an association between raised serum homocysteine and ischaemic stroke, similar to results obtained by Nura*et al,* 2006. In this study 34% of patients had hyperhomocysteinaemia with a mean serum homocysteine levels of (mean ± SD): 1.62±0.35µmol/L vs. 1.30±0.02µmol/L) for study patients and controls respectively. This is similar to the finding of nura*et al,* 2006 in Maiduguri were serum homocysteine level of (mean ± SD: 20.8 ± 10.2µmol/L vs. 13.1 ± 4.5µmol/L) for patient and controls was reported. Their reported value was higher than this study and may be attributable to the method used. This study used ELISA method while they used flurescence polarization immunoassay, specificity and sensitivity, secondly the pattern of vitamin B_{12} of the patient is much lower explain the role of vitamin B_{12} in homocysteine metabolism. The relatively high homocysteine concentrations we observed in many of the 100 total subjects who were involved in this study and which confirm our previous findings in northern Nigeria by Nura*et al*this is probably as a result of inadequate vitamin B_{12} nutrition, since the serum levels of this vitamin was also low in about 81% is the cases. In addition to nutritional factors, genetic variability may be another important determinant of plasma homocysteine levels. Thus, variability in the prevalence of genetic mutations of the enzyme methylene tetrahydrofolate reductase and varied practices between countries regarding fortification of dietary flour with folic acid could explain the hyperhomocysteinaemia. We did not find any difference in mean plasma homocysteine between males and females in the patients in contrast to what Nura*et al* found in Maiduguri (Nura*et al,* 2006) probably because females tend to have better life style in North Western Nigeria than North East Nigeria.

Parnetti*et al.*in 2005 studied 161 consecutive patients with first-ever ischemic stroke classified using TOAST criteria and 152 neurologically healthy controls to assess the association between risk of stroke and increasing values of plasma homocysteine and the interaction between the mild hyperhomocysteinaemia and conventional vascular risk factors. Hcy was elevated in all stroke subtypes with mean (±SD) values of 13.0 ± 2.5 µmol/l in patients with cardioembolic disease, 13.9 ± 5.4 µmol/l in those with small vessel diseases, 15.5 ± 6.8 µmol/l in cases of undetermined stroke, and 17.8 ± 13.5 µmol/l in patients with large vessel disease. Mean homocysteine level was 8.10 µmol/l (SD = 2.5) in controls. They suggested that mild hyperhomocysteinaemia is confirmed to have a significant role as risk factor as an etiological factor been stroke subtypes (Tonder*et al*, 1990).

Although this study didn't sort out patients by their stroke subtypes, result is similar tothat of Parnetti*et al.*

In a prospective study, Perini et al. measured homocysteine plasma levels in stroke patients in order to investigate possible correlations of homocysteine with stroke severity and clinical outcome. The plasma level of hcy was neither an independent determinant for stroke severity nor for patient's outcome by the Barthel index. Mean plasma homocysteine of both ischemic and hemorrhagic stroke was significantly higher than in controls ($P<0.05$). Homocysteine in the acute phase of stroke was not associated with stroke severity or outcome. Higher levels were associated with higher risk of small artery disease subtype of stroke. They did not demonstrate that patients with high hcy levels in the acute phase of ischemic stroke have a worse outcome. This study of serum levels of homocysteine and severity in the acute phase of ischemic stroke, but not outcome of demonstrated elevation of plasma hcy is to the findings of perini*et al*. in the acute phase of stroke.

In 2007, Haapaniemi*et al.* measured plasma hcy levels in 102 consecutive stroke patients on admission and at 1 week, 1 month, and 3 months after stroke and only once in 102 control subjects. Compared with controls, plasma hcy levels in patients were significantly lower on admission but not at later time points, with levels increasing by week and remaining at this level for 3 months. They proposed that this phenomenon was due to acute phase response reflecting increased synthesis of acute-phase proteins. However, they were unable to identify any correlation between CRP concentration in the acute stage of stroke and hcy levels that corroborates earlier results (Lee*et al*, 2002).

This study was in contrast to what Lindgren *et al* and Nura*et al*, reported that the concentration of plasma hcy did not differ between cases and controls in the acute phase, and plasma hcy levels were in fact higher in the convalescent period following stroke; which compare to glew*et al* findings.

The mechanism by which hcy could influence the severity of tissue damage caused by ischemia is not clear. El Kossi and Zakharyin 2000 proposed that homocysteine could be responsible for free radical generation and this way lead to the injury during acute phase of stroke. Similar conclusion was proposed by Al-Obaidi*et al.* in 2000. A number of mechanisms have been proposed to link total homocysteine to stroke. These include impairment of endothelial functions, endothelial desquamation, oxidation of LDL, increased monocyte adhesion to the vessel wall, impaired vascular response to nitric oxide, and thrombotic tendency mediated by activation of coagulation factors and platelet dysfunction.

In this study we found that the levels of serum vitamin B_{12} in patientswere significantly lower than that of their age-matched controls, 81% of our stroke patients had low vitamin B_{12}, with mean (SD) serum vitamin B_{12} of stroke patients was found to be 133.15(100.92). This is in contrast to what Hoseinali*et al* found in Iran in which no significant difference statistically between vitamin B_{12} of cases and controls was found a383.96 (275.22) (Hoseinali*et al* 2011). This hypovitaminosis B_{12} we found in our stroke cases was probably the cause of significant hyperhomocysteinaemia in them, because vitamin B_{12} is an essential cofactor in the metabolism of homocysteine (Yoo*et al* 1998). We also found a significant negative correlation between serum homocysteine and serum vitamin B_{12} of cases (p= 0.04, r = -0.198) explanatory being that low vitamin B_{12} causes hyperhomocysteinaemia. Other studies have found a direct relation between even mild or moderately elevated serum homocysteine levels and the risk of both coronary artery and cerebrovascular thrombosis (Yoo*et al*, 1998); Cobalamin deficiency, which elevates plasma homocysteine, might, thus, be a cause of otherwise unexplained ischemic stroke or cranial artery dissection (Penix, 1998; and Whyte *et al*, 2012). Total homocysteine levels are inversely related to the levels of cobalamin consumed in the diet (Siri*et al*,1998). Thus, cobalamin deficiency might increase the risk of cerebral infarction by increasing serum homocysteine levels.

Current study found that the levels of serum zinc in patients were significantly lower than that of their age-matched controls of the same population with levels of ≤52.26 µg/dL in 46% of these patients had low zinc levels. The association between low zinc levels and stroke has been earlier reported.Arctibatt*et al* in that study carried out in Michigan State University, East Lansing, USAout of the 224 stroke patientsanalyzed (mean age 67 years), 35.7% patients had low zinc levels (65 µg/dL), the zinc level in this study was lower than theirs may bedue to lower socioeconomic status in our patients and therefore consumption of zinc is lower in this environment. There are studiesthat had evaluated the role of zinc in cerebral ischaemia, though the mechanism is still unclear (Mushin *et al,* 2010). Mushin *et al* suggested that low zinc levels may be in fact a risk factor for stroke, in that studies serum samples from 256 ischaemic stroke patients and 180 healthy, age and sex matched controls. The concentration of zinc was significantly lower in stroke patients (p = 0.001) as compared to normal subjects. Zinc may represent an independent risk factor for stroke and therefore a possible target for prevention. Other studies that evaluated zinc in cerebral ischaemia postulated that it would be neurotoxic, neuroprotective or both. (Galasso*et al*, 2007). Additional studies are needed to further examine the role of zinc in the pathogenesis of stroke.

The reference interval we generated from our study for serum homocysteine was 0.90-1.70µmol/l which different from what Nura*et al* used in Maiduguri most likely because of difference in methodology.

The reference interval we established for vitamin B_{12} was similar to that reported by the US population with the lower limit almost the same while the upper limit was larger for the US population which was found to be 200pg/ml-853pg/ml. (Selhub*et al*, 1999)this may probably because of better nutritional status of US population ..

The reference interval found in this study for zinc was lower than that of the US population which was found to be 80-120 µg/dl(teitzpg, 2302). Nutrition may contribute the low value.

No significant relationship statistically between hcy level and severity of stroke, which is similar to what Haapaniemi*et al* reported. The study did not show relationship significant statistically between low vitamin B_{12} and stroke severity and to the best of our knowledge yet to find similar studies.

CHAPTER SIX

6.0 CONCLUSION AND RECCOMMENDATIONS

6.1 SUMMARY

In summary, the main findings were as follows

(i) Hcy serum levels in the acute phase of ischaemic stroke (within 72 hours) were significantly higher than that of controls

(ii) Serum vitamin B_{12} and zinc in the acute phase of ischemic stroke (within 72 hours) were significantly lower than that of controls.

(iii) There was no relationship between B_{12} vitamin homocysteine and zinc and severity of stroke.

(iv) There was significant differences in B_{12} vitamin and zinc were observed between patients and controls.

6.2 CONCLUSION

We can conclude that elevated serum homocysteine level, low serum vitamin B_{12} and zinc may be an independent risk factor for ischemic stroke. But, because this study was a case control one, we could not rule out the possibility of acute phase response being responsible for the elevation of serum hcy level in acute stroke patients. More prospective and population based studies are needed to define whether elevated homocysteine level, low vitamin B_{12} and zinc is an independent risk factor for cerebrovascular diseases or stroke in our environment. Early management of hyperhomocysteinaemia, low vitamin $B_{12}$and zinc may be an effective way of decreasing the incidence of stroke in our environment. Vitamin B_{12} and zinc supplements may be beneficial to patients at risk.

6.3 RECOMMENDATIONS

(i). Homocysteine determination levels for patients with an ischemic stroke is safe and cheap and may be of prognostic significance. It may be useful to introduce determining the homocysteine levels inischaemic stroke patients with vascular disease and thrombosis, or ischaemic stroke at a young age and with family history of premature atherosclerosis.

(ii). A large epidemiological prospective study is necessary to determine the importance of serum zinc levels in patients with ischemic stroke and to investigate whether low zinc levels are associated poor long-term outcomes.

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APPENDIX I

PATIENT INFORMATION FORM

Serial No……. Hospital No…………… Date……………. Age……. Phone no……………

This study is on " Evaluation of Serum Levels of Homocysteine vitamin B_{12} and zinc in patients with Acute Ischaemic Stroke" will be carried out by me, Dr Hafsatu Maiwada Suleiman an MSc candidate of Department of Chemical Pathology of ABU Zaria

It involves collection of blood from acute ischaemic stroke patients that have been brought to ABUTH Zaria to analyze homocysteine vitamin B_{12} and zinc and neurological examination will also be done.

You are free to participate or decline from the study without any consequences.

Your safety shall be given utmost priority.

The study will help in improving the active management of patients with acute ischaemic stroke.

The data from the study will be treated with strict confidentiality.

The test will be at no cost to you and if any abnormality is detected you shall be contacted through the phone numbers provided.

If you have agreed to participate in this study kindly sign the attached form.

Thank you

Dr Hafsatu Maiwada Suleiman

08037013611

APPENDIX II

WRITTEN INFORMED CONSENT

I ………………………………………………. of ………………………………… (Address) Have agreed to participate in this study of topic: Evaluation of serum levels of homocysteine vitamin B_{12} and zinc in patients with acute ischaemic stroke in Zaria ".The full procedure before and after the test has been explained to me. I understand that a sample of my blood will be taken for test. I give this consent willfully without being subjected to any pressure

Participants Name ……………………………….. Signature……………..

Patients care giver's name and relationship………………………… signature……………..

Researchers name…………………………………. signature……………..

APPENDIX III

QUESTIONARE

PERSONAL INFORMATION

Hospital no…………. Phone no………….. Serial no………… Age………sex…………

Ethnic group………… Occupation………… Religion…………Height……….

Weight………… BMI………….. SBP……………..DBP……………………..

Marital status

i. Widowed…………
ii. Divorced………….
iii. Single…………….
iv. Married…………
v. Others…………..

Level of education

i. Graduate/postgraduate……………..
ii. Undergraduate…………………
iii. NCE/Diploma………………
iv. Secondary…………………..
v. Primary……………….
vi. Quranic………………
vii. None………………

Is patient on methotrexate, tamoxifen, L- DOPA, phenytoin or bile acid sequestrants? ……………..

Time of onset of present illness ______________________________

NEUROLOGICAL EXAMINATION by

A) National Institute Of Health Stroke Scale (NIHSS)

1 a) Level of consciousness	0 Alert 1 Not alert, but rousable with minimal Stimulation 2 Not alert: requires repeated stimulation to attend 3 Coma
1 b) Ask patient the present month and his/her age	0 Answers both correctly 1 Answers one correctly 2 Both incorrect
1 c) Ask patient to open and close eyes	0 Obeys both correctly 1 Obeys one correctly 2 both incorrect
2. Best gaze (only horizontal eye movement)	0 normal 1 partial gaze palsy 2 forced deviation
3. Visual field testing	0 No visual loss 1 partial hemianopia 2 complete hemianopia 3 bilateral hemianopia (blind including cortical blindness)
4. Facial paresis (ask patient to show teeth or raise eye brows and close eyes tight	0 Normal symmetrical movement 1 Minor paresis; flattened nasolabial fold, asymmetry on smiling 2 partial paralysis (total or near total paralysis of lower face) 3 complete paralysis of one or both sides (absence of facial movements in the upper and lower face).
5. Motor function or the arm (right and left) Right arm Left arm	0 Normal (extends arms 90° (or 45°) degrees for10 seconds without drift 1. Drift 2. Some effort against gravity 3. no effort against gravity 4. No movement 9 untestable (joint fused or limb amputated)
6. Motor function of the Leg (Right and Left) Right leg Left leg	0. Normal (Holds leg 30 degrees position for 5 seconds) 1. drift 2. some effort against gravity 3. no effort against gravity 4. No movement 9 unstable (joint fused or limb amputated)
7. Limb Ataxia	0 No ataxia 5 seconds) 1 present in one limb 2 present in two limbs
8. Sensory (use pin prick to test –arms, leg, trunk and face- compare side to side).	0 Normal 1 mild to moderate decrease in sensation 2 severe to total sensory loss
9. Best language (Describe picture, name items, read sentences)	0. No aphasia 1. mild to moderate aphasia 2. severe aphasia 3 .mute
10. Dysarthria (read several words)	0 Normal articulation 1 mild to moderate slurring of words 2 unintelligible or unable to speak 9 intubated or other physical barrier
11. extinction/inattention	0 normal 1 mild 2. severe

NIHSS SCORE:-------------------- INTERPRETATION FOR NIHSS Score

0 = No Stroke Symptoms
1-4 = Minor Stroke
5-15 = Moderate Stroke
16-20 = Moderate to Severe Stroke
21-42 = Severe Stroke

SIRI RAJ STROKE SCORE

(a) Level of consciousness at onset

0= Alert 1= Drowsy/stupor

2=Coma

(b) Vomiting 0= Absent 1= Present

(c) Headache 0=Absent 1= Present

(d)Atheroma markers (History of DM, angina, intermittent claudication)

0= Absent 1=Present

SRScore (2.5×(a)__)+(2×(b)__)+(2×(c)__)+(0.1×DBP___)−(3×(d)__)−12

TOTAL=

Summary: ☐ Ischaemic stroke (score below −1)

☐ Haemorrhagic stroke (score above 1)

☐ Equivocal (score between 1and -1)

Siriraj score ________________________

BIOCHEMICAL RESULTS

Serum Homocysteine (µmol/l) _________________

Serum vitamin B_{12} (pg/ml) ___________________

Serum zinc (µg/dl) ____________________