COMPARATIVE STUDY OF SERUM SELENIUM LEVELS IN ESSENTIAL HYPERTENSIVE AND NORMOTENSIVE ADULTS AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA, ZAMBIA

By

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A dissertation submitted to the University of Zambia in partial fulfillment of the requirements for the award of the Degree of Master of Science in Biochemistry

THE UNIVERSITY OF ZAMBIA

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DECLARATION

I **Angela Chiti Chisulo** do hereby declare that this dissertation is original representation of my own work and in submitting it for my Master of Science in Biochemistry program, further attest that it has not been submitted to another University in part or whole for the award of any degree, diploma or any other qualification. All published work from other sources which have been incorporated has been properly acknowledged.

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CERTIFICATE OF APPROVAL

This dissertation of Angela Chiti Chisulo has been approved as fulfilling the requirements for the award of the degree of Master of Science in Biochemistry by the University of Zambia.

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SUPERVISOR'S CERTIFICATE OF COMPLETION

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ABSTRACT

In Zambia, essential hypertension is one of the commonest and prevalent non-communicable diseases. In the current medical literature it is not clear on the serum selenium levels among essential hypertensive adults in Zambia despite evidence in literature of its role in development of essential hypertension. The present study investigated serum selenium levels in essential hypertensive adults attending clinic 5 at the University Teaching Hospital Lusaka Zambia. We hypothesized that serum selenium levels were significantly lower in this population and was a risk factor for developing essential hypertension. An analytical cross-sectional design was applied to a total of 245 participants. These were composed of 126 essential hypertensive patients and 119 healthy normotensive adults of both sexes. Only participants who met the inclusion criteria and agreed to take part in the study by signing consent forms were enrolled. Blood was collected for serum levels of Selenium, glucose, urea, creatinine and electrolytes. Student t-test was used to compare the mean serum selenium levels between hypertensive and normotensive participants as data was normally distributed. Lower levels of serum Selenium were observed in essential hypertensive adults (0.093 ± 0.048 mg/L) than in healthy normotensive adults (0.109± 0.047) and this was statistically significant (*p*-0.0001). Linear regression results showed no significant relationship off Selenium levels with age (*p*-0.255), BMI (*p*-0.232), systolic blood pressure (*p*-0.195) and diastolic blood pressure (p-176).The present study found that the mean serum selenium levels in hypertensive participants were significantly lower compared to normotensive participants. However, serum selenium levels were not significantly related to blood pressure hence serum selenium levels may not be a risk factor for development of essential hypertension in this population. Nevertheless, more studies in the same geographical area are needed to confirm this.

Key words: Hypertension, Essential hypertension, Selenium, Oxidative stress and Reactive Oxygen species (ROS)

DEDICATION

My father Dominic Able Chisulo (MHSRIP) and mother Josephine Ngosa.

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ABBREVIATIONS

AAS	Atomic Absorption Spectroscopy
AFS	Atomic fluorescence spectrometry
BP	Blood pressure
DNA	Deoxyribonucleic acid
GART	Golden Valley Agricultural Research Trust
GC	Gas chromatography
GPx	Glutathione Peroxidase
GSH	Reduced form of Glutathione
GSSG	Oxidised form of Glutathione
ICP-MS	Inductively coupled mass spectrometry
ICP-AES	Inductively coupled atomic emission spectrometry
NO	Nitric oxide
NAA	Neuron activation analysis
Pb	Lead
ROS	Reactive oxygen species
SOD	Superoxide Dismutase
Se	Selenium
UTH	University Teaching Hospital
WHO	World Health Organization
XRF	X-ray fluorescence spectrometry

DEFINITION OF KEY OPERATIONAL TERMS

Low blood pressure	blood pressure below 90 mmHg systolic and below 60 mmHg diastolic.
Normotensives (normal blood pressure)	Participants in the study without hypertension systolic pressure ≤ 120mm Hg and diastolic pressure Ò80 mmHg)
Adults	Individuals with age ranging from 18-65 years old
Hypertensive (high blood pressure)	Participants in the study with blood pressure that comes above 140 mmHg (systolic) and/or 90 mmHg (diastolic)
Essential hypertension	participants with high blood pressure with unknown cause

CHAPTER ONE: INTRODUCTION

1.1 Background

Emergency of serious diseases caused by many infective organisms as well as nonspecific illnesses, has led to the need to know about the role of essential minerals in human health. Lifestyle changes and nutritional habits have equally contributed to speculation about the role of essential elements in diseases. Selenium (Se) in particular has attracted attention for its role in cardiovascular diseases (Stranges et al., 2011). It has also attracted attention in other diseases and particularly in HIV infected patients (Chisenga., 2014) as well as in cancer patients. A number of reviews abound focusing on the relationship between Selenium and hypertension; thus indicating its possible role in this condition. Hypertension is a major global health problem with its accompanying morbidity and mortality (Nguyen et al., 2013). Hypertension, defined as a systolic blood pressure Ó140 mmHg and/or a diastolic pressure Ó90 mmHg, is one of the most common chronic diseases (Bolívar, 2013). Essential hypertension or primary hypertension is known to account for 95% of all hypertensive cases compared to 5% due to the secondary causes of hypertension (Montezano and Touyz, 2012). Essential hypertension refers to the majority of people with sustained high blood pressure for which there is no obvious, identifiable cause (Subash et al., 2010). However, oxidative stress which is the imbalance between reactive oxygen species (ROS) production and elimination of antioxidant has been established to be one of the risk factors for development of essential hypertension (González et al., 2014).

In the cardiovascular system, ROS play a physiological role in controlling processes that contribute to endothelial dysfunction and cardiovascular remodeling in hypertension and other cardiovascular diseases (Montezano and Touyz, 2012, Handy et al., 2016). There are various antioxidant mechanisms that the body uses to fight off these ROS which includes enzymes and vitamins (Baradaran et al., 2014). Selenium found incorporated in some antioxidant enzymes (glutathione peroxidase (GPx), thioredoxin reductases) has generated a lot of interest about its possible role in aetiology of cardiovascular diseases (Stranges et al., 2011, Rayman., 2012, Benstoem et al., 2015). Selenium is a co-factor of the antioxidant enzymes which are crucial in prevention of oxidative stress and normal antioxidant signalling (Preedy, 2015).

Selenium levels in Zambian soils have been reported to be low (Melse-Boonstra et al., 2007). Selenium dietary intake is dependent on the food and amount taken in from animal and plant

sources. Plants obtain their Selenium from the soil and so Selenium plant content depends on the soil content (Huang et al., 2013).

Selenium is usually gotten through vegetables in the diet and the apparent reported low levels in the soil could explain the deficiencies in the Zambian population (Melse-Boonstra et al., 2007).

In Zambia , localized studies have shown that the prevalence rate for hypertension is 34.8 percent in the urban district of Lusaka, Zambia (Goma et al., 2011). A recent observational study found that the crude prevalence of hypertension was 23.1 percent among adults presenting to primary health clinics in rural Zambia (Yan et al., 2015). In Kasama district the prevalence of hypertension was found to be 30.3 percent while in rural Kaoma district it was 25.8 percent (Mulenga et al., 2013). The levels of hypertension in Zambia are structurally higher in urban than in rural settings. These findings are similar to what has been reported in most African nations, levels of hypertension are higher in urban settings than in rural settings (van de Vijver et al., 2014). This calls for measures to be undertaken in order to manage and treat hypertension as it is clearly a national problem from the findings of these studies.

In Zambia, there is paucity of data with regards the role of Selenium in essential hypertension. In the present study serum Selenium levels were determined by Graphite furnace atomic absorption in normotensive and essential hypertensive adults at UTH.

1.2 Statement of the problem

Hypertension affects one billion people worldwide and about nine million people die every year (WHO, 2015). In Zambia, 8 percent of deaths are as a result of cardiovascular diseases (WHO, 2014) and approximately 1162 deaths that occur annually were reported to be hypertension related.

Selenium as a co-factor to some antioxidant enzymes has generated a lot of interest about its possible role in aetiology of cardiovascular diseases (Stranges et al., 2011). Many studies have been done to ascertain the possible role that Selenium plays in aetiology of cardiovascular diseases and in particular hypertension with conflicting findings (Kuruppu et al., 2014, Nawrot et al., 2007). Selenium is a microelement that is usually obtained from soil by plants. Animals then obtain Selenium as they feed on these plants and so Selenium concentration levels in humans is mostly dependent on geographical locations and this could possibly explain these reported different findings in the role of Selenium in hypertension (Kuruppu et al., 2014,Stranges et al., 2011, Nawrot et al., 2007). Zambia has reportedly low levels of Selenium levels in soils (Melse-Boonstra et al., 2007). The reported low levels of Selenium in soil in Zambia called for more studies to investigate what could be happening in this population and the possible role this played in hypertensive disorders.

This study therefore addressed the question as to whether there were differences in serum Selenium levels in normotensive and essential hypertensive adults at University Teaching Hospital (UTH) in an effort to address the problem of essential hypertension in Zambia.

1.3 Justification of the Study

Prior to this study in Zambia, little or no information had been documented on the role of Selenium in hypertension in the adult population. The conflicting reports on the role of Selenium in hypertension, made it very difficult to extrapolate findings from other countries. This study provided evidence-based novel data on Selenium level in essential hypertensive patients in relation to normotensive individuals in Zambia. In addition, the information generated from this study would be useful to policy makers and medical practitioners in the management of hypertensive disorders. The results of the study would be used to formulate further hypothesis that would investigate Selenium in hypertension beyond the scope of this study.

1.4 Research Question

Are there differences in serum Selenium levels in essential hypertensive and normotensive adults at the University Teaching Hospital, Lusaka, Zambia?

1.5 General Objective

To evaluate serum Selenium levels in normotensive and essential hypertensive adults attending filter clinic and clinic 5 at the University Teaching Hospital.

1.6 Specific Objective

I. To determine and compare the serum Selenium levels in essential hypertensive and normotensive adults at University Teaching Hospital.

II. To establish the relationship between age, BMI and serum selenium levels in Zambian adults attending clinic 5 at UTH

III. To establish the association between serum Selenium levels and essential hypertension in Zambian adults.

CHAPTER TWO: LITERATURE REVIEW

2.1 Background

Hypertension or raised blood pressure is one of the key risk factors for cardiovascular disease. It already affects one billion people worldwide (WHO, 2015). It leads to myocardial infarction and cerebral vascular injuries. Researchers have estimated that nine million people die every year from hypertension (WHO, 2015). Essential hypertension or primary hypertension accounts for 95% of all hypertensive cases compared to 5% due to secondary causes of hypertension (Montezano and Touyz, 2012). Essential hypertension is high blood pressure with no identifiable cause (Subash et al., 2010).

Oxidative stress, which is the imbalance between reactive oxygen species (ROS) production and antioxidants elimination, has been established to be one of the risk factors in aetiology of essential hypertension (González et al., 2014). Reactive oxygen species are generated from oxygen (O_2) during normal function of mitochondria as a result of electron transport chain. Oxygen can readily oxidize other molecules (Montezano and Touyz, 2012). Most intracellular ROS are derived from superoxide ($O_2^{\bar{1}}$) generated by the one electron reduction of O_2. Superoxide dismutase (SODs) converts superoxide to hydrogen peroxide (H_2O_2) (Sena and Chandel, 2012). In the cardiovascular system, ROS play a physiological role in controlling processes such as: endothelial function, vascular tone and cardiac function (Montezano and Touyz, 2012). All of these processes contribute to endothelial dysfunction and cardiovascular remodelling in hypertension and other cardiovascular diseases (Handy et al., 2016). Selenium exerts antioxidant function as a component of ROS-detoxifying selenoproteins such as Glutathione Peroxidase (GPx) or Thioredoxin reductase (González et al., 2014). Selenium-dependent enzymes such as GPx convert H_2O_2into water (H_2O) (Berg et al., 2002). They maintain Nitric oxide (NO) in its reduced form hence protecting against oxidative stress (Nawrot et al., 2007). Nitric oxide is the endothelium-derived relaxing factor (EDRF). Nitric oxide role is very important in the normal activity of cells (Afanas'ev., 2010). Nitric oxide relaxes the smooth muscles of blood vessels blood hence helping in regulation of blood pressure (Murray et al., 2003). Nitric oxide can also react with other radical elements such as superoxide to generate peroxynitrite (Predonzani et al., 2015). Glutathione Peroxidase is among a number of antioxidants which reduces peroxynitrite into NO and also helps in maintaining the release of NO by the endothelium (Wolin., 2011). Selenium therefore as co-factor and component of GPx maintains membrane integrity and limit the propagation of oxidative damage to lipids, lipoproteins, and deoxyribonucleic acid (DNA) (Mistry et al., 2012). It is through this

mechanism that Selenium has been thought to be possibly associated with essential hypertension. Selenium plays a role in prevention of oxidative stress as a cofactor of antioxidant enzyme GPx as shown in Figure 2.1.

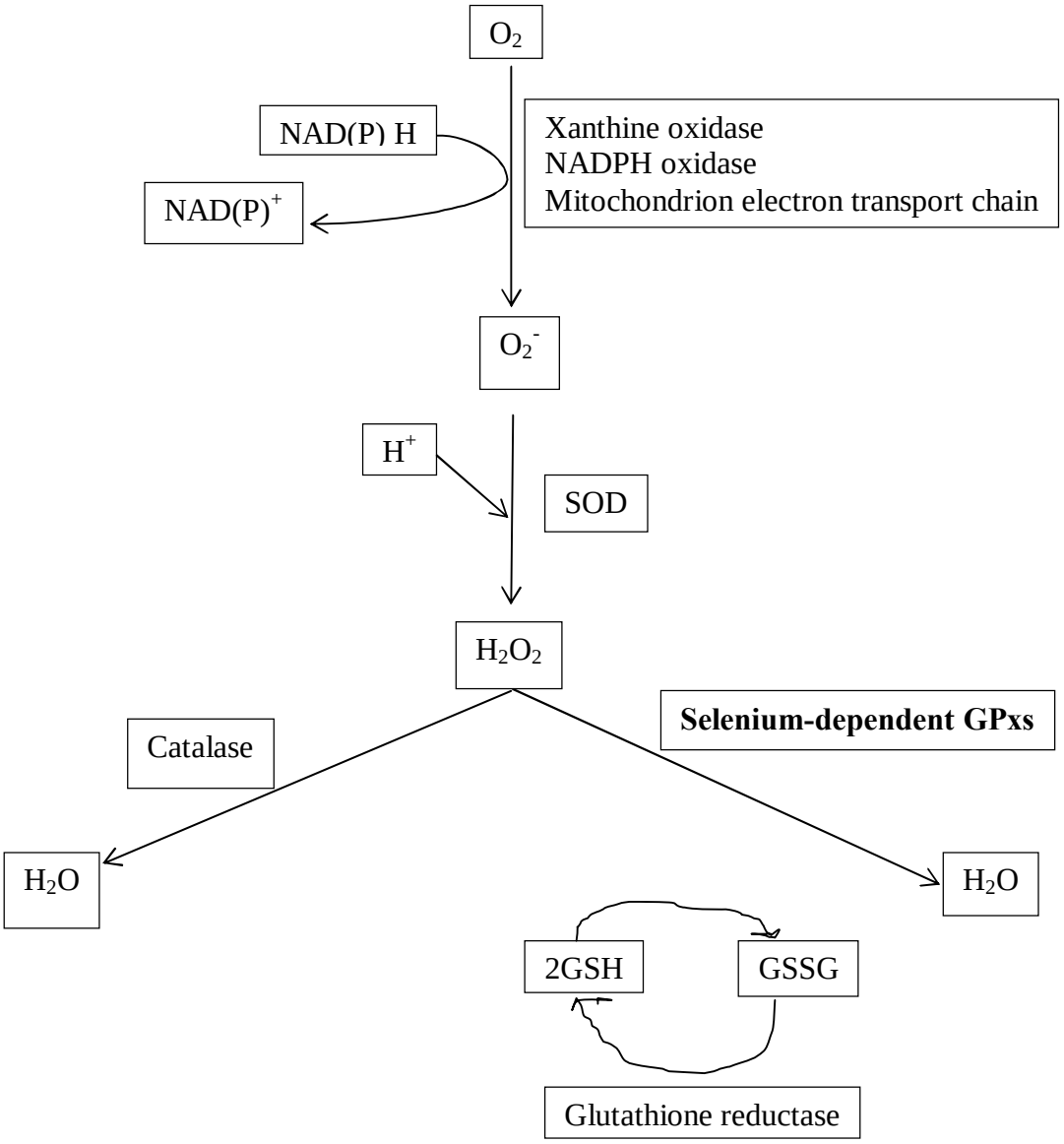

Figure 2.1 Relationship between Reactive Oxygen Species and Selenium Antioxidant Enzymes (Mistry et al., 2012)

In order to establish if there was a possible relationship between Selenium and essential hypertension; there was a need to determine Selenium levels in both normotensive and essential hypertensive adult. Trace element concentrations in tissue are used as indicators as direct determination of metals in vivo in organs is not possible (Ļuparigova and Stafilov, 2013). Indirect determination of metals in accessible tissue can point at the presence of elements (Ļuparigova and Stafilov, 2013). Selenium can therefore be measured by using a variety of media including: plasma or serum, whole blood, red cells, platelets, urine, hair, nails and by

indirect measurement using the activity of the enzyme GPx as an indicator of Selenium status (Mehdi et al., 2013). Plasma or serum Selenium is one of the most commonly used biomarkers of Selenium levels because it is relatively easy to obtain trace element-free collection tubes (Fairweather-Tait et al., 2011). The current study therefore measured Selenium levels in serum.

Analytical methods that are used in determination of Selenium include: Neuron activation analysis (NAA), X-ray fluorescence spectrometry (XRF), Atomic fluorescence spectrometry (AFS), Gas chromatography (GC), Inductively coupled mass spectrometry (ICP-MS), and Inductively coupled atomic emission spectrometry (ICP-AES) (Fordyce, 2013).Serum Selenium can also be detected by electrothermal atomic absorption Spectrometry (Ļuparigova and Stafilov). All these methods are highly sensitive methods in detection of Selenium in serum or any other biological samples.

Serum Selenium can also be measured by Graphite furnace atomic absorption spectrophotometry (Askari et al., 2015). In the present study determination of Selenium levels was carried out by both Graphite furnace atomic absorption and classical flame atomic absorption spectroscopy. However, classical flame AAS techniques do not have sufficiently low detection limits (Koirtyohann and Morris., 1986). This implies that they do not easily detect low levels of Selenium in biological samples. Graphite furnace atomic absorption spectroscopy on the other hand has a high sensitivity in detection of Selenium (Lewis., 1988). However, both methods were used in the present study after comparison of the results of the analysis of the serum samples by both these techniques gave same results.

2.2 A Global Review on selenium levels in hypertension

Globally, systematic reviews of literature have found that the association of Selenium levels with hypertension has been inconsistent (Kuruppu et al., 2014).Some researchers have reported Selenium deficiency (Nawrot et al., 2007) or its excess (Laclaustra et al., 2009) to be associated with hypertension. Others on the other hand have reported no association between Selenium and hypertension (Kuruppu et al., 2014, Nawrot et al., 2007, Stranges et al., 2011,Laclaustra et al., 2009). Some studies have even reported no differences in selenium levels in normotensive and essential hypertensive adults but in pregnant women (da Silva et al., 2017)

A study on Croatian men reported that reduction in Selenium levels in the study population lead to increased systolic and diastolic blood pressure (Teliġman et al., 2001). However, the reported association could have been due to the interaction between Lead (Pb) and Selenium (Teliġman et

al., 2001). Interaction of Lead with Selenium was reported to affect the absorption and retention of Selenium leading to lowered concentrations (Teligman et al., 2001).

That study had only male participants and thus did not show whether the selenium levels were different in women. The current study however tried to establish selenium levels in women and expected some differences compared to men, due to occupational difference among the two groups in the Zambian scenario.

A study in Belgium reported that higher blood Selenium levels were associated with lower systolic and diastolic blood pressure levels in men but not women (Nawrot et al., 2007). The information from that study was in line with what other researchers had reported on the role of Selenium levels in reducing the risk of developing hypertension. Therefore the current study focused on the Zambian population to establish if there were differences in selenium levels in normotensives and essential hypertensive adults.

In a study done by Shargorodsky et al (2010), Selenium was found to have beneficial effect on blood pressure in people with multiple cardiovascular risk factors (Shargorodsky et al., 2010). However, that study looked at the effect of combined supplementation of Selenium with other antioxidants. The results of that study could not be generalized as the effect of supplementation of Selenium in hypertension as other antioxidants also played a role in the observed results. In order to establish whether Selenium supplementation was beneficial in hypertension, a study would be required in which participants were only given Selenium as an intervention. In line with this, the current study sought to assess the role of Selenium in hypertension ruling out other possible confounders.

Other studies have reported that excess concentration levels of Selenium were associated with the risk of hypertension. One example of such a study was a cross-sectional analysis of serum Selenium and hypertension in the United States of America (USA) (Laclaustra et al., 2009). That study reported that higher Selenium levels were associated with a higher prevalence of hypertension in both men and women. However, the USA has been reported to have high Selenium content in the soil which could be a possible explanation for the findings of that study but may not be the case in Zambia. It was on the basis of these conflicting reports that a study on the Zambian scenario was needed to fill the knowledge gap, especially, in the absence of any documented studies of this nature in Zambia currently to my knowledge.

A study conducted in males in Southern Italy found no association of Selenium levels with hypertension in either cross-sectional or longitudinal analyses (Stranges et al., 2011). However,

the results of these studies might not be representative of Zambia due to the fact that they were conducted in Europe and the USA where fortification of foods with Selenium is common practice (Sacco et al., 2013, Giacosa et al., 2014, Benstoem et al., 2015).

2.3 Regional review of selenium levels in hypertension

In Africa few studies have been conducted to find out if there was a relationship between Selenium levels in normotensive and essential hypertensive adults**.** A study conducted in Nigeria with 103 hypertensive and 88 healthy participants reported that there were differences in selenium levels between these two groups and that severity of hypertension was related to selenium levels (Babalola et al., 2007). However, due to different geographical positions of Zambia and Nigeria, the findings in Nigeria could not be used to interpret the rise in hypertensive cases in Zambia. It was therefore necessary to conduct a study that could give a clear indication of serum Selenium levels in essential hypertensive adults and normotensive adults in Zambia.

In Malawi it has been reported prevalence of Selenium deficiency in 500 healthy individuals was 88 % (Melse-Boonstra et al., 2007). Zambia and Malawi are close neighbours and so could have similar scenarios in terms of Selenium deficiency. The Golden Valley Agricultural Research Trust (GART) study which included Zambia alongside other African countries (Burundi, Niger, Nigeria and South Africa) reported that about more than half of the population in the study were Selenium deficient (Melse-Boonstra et al., 2007). That study also reported that Zambia had low soil Selenium content. Selenium is mostly obtained from dietary intake but this Selenium is initially taken up from the soil and concentrated by plants (Mistry et al., 2012). With the prevalence of Selenium deficiency at such a high level, it was important to find out whether the rise in the number of essential hypertensive individuals in Zambia could be associated with these levels of Selenium.

2.4 Local review on Selenium in hypertension

There has been no study to our knowledge conducted on the levels of Selenium in cardiovascular diseases and particularly hypertension in Zambia. A few studies that have been done so far in Zambia have looked at Selenium in association with HIV/AIDS (Melse-Boonstra et al., 2007). Other studies have looked at fortification of foods with Selenium and the benefits in infants (Gibson et al., 2011). The present study therefore provided the baseline information for future studies in Selenium in relation with hypertension in Zambia.

2.5 Summary and Interpretation of Literature review

The literature review showed that a number of researchers had shown relationships between Selenium levels and hypertension with conflicting findings. Some researchers had reported

associations of Selenium with cardiovascular disease such as hypertension whilst others found none. Even amongst researchers that found associations between Selenium and hypertension, there were conflicting reports on whether this association was due to excess levels of Selenium or low levels of Selenium. The literature also reviewed that Selenium levels in individuals was dependent on their geographical locations and that Zambia reportedly lies in a region with low Selenium content. None of the studies carried out in Zambia to our knowledge had looked at serum Selenium levels in normotensive and hypertensive individuals. However, many of studies that had been conducted on Selenium in Zambia were conducted in relation with its role in HIV/AIDS. The prevalence of hypertension has been on the rise despite advancements in understanding and treatment in Zambia. What this meant then was that there was a need to establish Selenium levels in individuals with essential hypertension and those without hypertension. From the review of the literature it was clear that many questions had not yet been addressed on hypertension in Zambia. A number of studies have been conducted in urban and rural Zambia trying to determine the prevalence of hypertension but few studies reported on what could be associated with this increase in hypertensive prevalence. Studying Selenium levels in normotensive and essential hypertensive adults was therefore an important undertaking whose results could be potentially useful as a point of reference.

This study therefore aimed at comparing serum selenium levels in normotensive and essential hypertensive adults at the University Teaching Hospital.

CHAPTER THREE: METHODOLOGY

This section of the dissertation dealt with the methodology that was used in conducting the study.

3.1 Study design

A cross-sectional study design was used to establish the relationship of serum Selenium levels in normotensive and essential hypertensive adults.

3.2 Study setting

The study was carried out at the University Teaching Hospital (UTH) which is the largest tertiary hospital in Zambia providing medical and surgical services for most of the population in Lusaka as well as referred cases from other provinces.

3.3 Target population

Hypertensive patients from clinic 5 and normotensives patients from Filter clinic aged 18-65 years that were attended to at UTH from May 2016 to May 2017.

3.4 Selection of study participants

Participants were selected based on being essential hypertensive or normotensive upon confirmation by a physician. Normotensive participants were individuals with normal blood pressure (systolic ≤ 120 mm Hg and diastolic pressure ≤ 80 mmHg) whilst essential hypertensive participants were participants with high blood pressure (systolic blood pressure Ó 140 mmHg and/or a diastolic pressure Ó 90 mmHg) with unknown cause. They were also selected based on the inclusion criteria and matched by age and sex. All the study participants underwent a thorough medical examination to determine their general health status and those who met the exclusion criteria excluded from the study.

3.5 Inclusion criteria for Normotensive Adults

- Adults of both sexes between 18 and 65 years of age
- Non-smokers
- Adults with normal blood pressure (systolic ≤ 120mm Hg and diastolic pressure ≤ 80 mmHg)
- Participants who were willing to take part in the study

3.6 Exclusion criteria for Normotensive Adults

- Pregnancy
- Adults who were obese (BMI >30 kg/m^2) were excluded from the study as it is one of the risk factors of hypertension.
- Diabetes mellitus
- Low blood pressure

3.8 Inclusion criteria for Essential hypertensive Adults

- Adults of both sexes between 18 and 65 years of age
- Non-smokers
- Adults with high blood pressure (systolic blood pressure Ó 140 mmHg and/or a diastolic pressure Ó90 mmHg).
- Participants who were willing to take part in the study

3.9 Exclusion criteria for Essential hypertensive Adults

- Pregnancy
- Individuals who were obese (BMI >30 kg/m^2) were excluded because obesity being a risk factor of hypertension would have been a possible confounder in the study.
- Diabetes mellitus
- Low blood pressure
- Secondary causes of hypertension such as coarctation of aorta, renal diseases e.g. polycystic kidney disease, Endocrine disorders e.g. hyperthyroidism, and Acromegaly were ruled out after review of patients files. Participants who met exclusion criteria were not included in the study.

3.10 Sampling method

The participants were systematically selected where every 3rd person was sampled and matched by sex and age.

3.11 Specimen collection, storage and analysis

Blood Pressure measurements

Measurement of systolic/diastolic blood was done using a mercury sphygmomanometer. Blood pressure readings were obtained by trained and certified nurses. Study participants first rested quietly in sitting position for 5 minutes and then 3 blood pressure readings were obtained consecutively using a mercury sphygmomanometer with a cuff placed on the bare right arm. The average of these readings was then recorded in the physical examination form.

Weight, height and BMI measurements

The weight of the study participants was also measured using a weight measuring scale Height was measured using a wall mounted height measuring scale. BMI was calculated by dividing the weight of study participants with the square of their height.

Collection and examination of urine samples

10 mls of urine samples was collected in urine containers for urinalysis. Immediately after collection the urine, urinalysis was done using urinalysis strips and results recorded on the physical examination form.

Collection of blood samples for examination

8mls of venous blood from the antecubital vein was collected from study participants in two aliquots into4mls plain vacutainers labelled A and B by qualified nurses. The participantôs ID number was clearly indicated on these vacutainer for the purpose of determining serum selenium level, urea, glucose, creatinine and electrolytes (Potassium and Sodium).

Storage of blood samples

Blood for Selenium analysis collected in plain vacutainers labeled A was immediately centrifuged at 400 rpm for 3 minutes to separate the serum from the blood. Serum samples were analysed immediately but if not analysed immediately it was stored at -20°C and analysed within 24 hours of collection.

Sample preparation for selenium analysis

- For the determination of Selenium, the samples were centrifuged at 400 rpm for 3 minutes to separate serum.

- 10mL Pd matrix modifier 5mL Mg matrix modifier was added to 0.8mL Triton X and 0.5mL 65% nitric acid are pipetted into a 100 mL volumetric flask. The volumetric flask was filled with deionised water.
- 0.5 ml of serum was diluted to 10ml with deionized Water
- 0.1% Acetic acid was added to the samples to denature the proteins and 0.1% Triton X to reduce surface tension. The dilution factor was 20.

Method: Determination of selenium in serum by flame atomic absorption spectrometry and by Graphite Furnace atomic Absorption Spectroscopy

Calibration

Calibration standards were prepared from Multi ï Element Calibration standards traceable to NIST in intermediate ranges of:

0.025/ 0.05/ 0.1/ 0.15/ 0.2/ 0.25 ppm from 1ppm (1000ug/L) stock standard.

A linear Calibration curve was obtained with Correlation Coefficient $R^2 = 0.997$

After the Reagents were added, the samples were ready to be analyzed using AA by both flame atomic absorption and graphite furnace atomic absorption. The measurements were performed with the ANALYTIK JENA ï ContrAA700 AAS.

Table 3.1 Properties of Selenium for flame atomic absorption spectroscopy.

ELEMENT	WAVELENGTH (nm)	FLAME TYPE	GAS FLOW RATE L/h	BURNER HEIGHT (mm)
Selenium	196.0267	Air/C_2 H_2	100	9

Table 3.2 Properties of Selenium for graphite furnace atomic absorption Spectroscopy

Element	Wavelength (nm)	Slit width	Background correction	Graphite furnace	Injection time
Selenium	196.0267	0.5 nm	Zeeman	Pyrolytic-coated	3s

Sample preparation and analysis for urea, creatinine, random blood sugar and electrolytes

The blood samples collected in plain vacutainers labeled B were centrifuged at 3000 rpm for 3 min and two aliquots of serum samples were put in vacutainer serum glass tubes labeled a and b. One of these aliquots (a) was immediately analysed for urea, creatinine, and electrolytes. The other aliquot (b) was analysed for random blood sugar.

Analysis for creatinine, urea, electrolytes and random blood sugar

Creatinine concentration was determined using the Jaffe method in which creatinine reacts with picric acid at an alkaline pH forming a yellow complex and absorbance at 520 nm to 800 nm using the Beckman coulter 480 clinical chemistry analyser. The Beckman coulter automatically computerized the concentration of creatinine. The concentration of urea was determined using the Beckman coulter analyser 480. The concentration of the urea was proportional to the absorbance at 340 nm due to disappearance of NADH. The analyser uses a method based on the Talke and Schubert enzymatic method. The concentration of electrolytes ï sodium and potassium was determined using an ABX Pentra 400 which gave automated results.

The Beckman coulter procedure was used to determine random blood sugar and this method was based on the method by Stein in which glucose was phosphorylated to glucose-6-phosphate by hexokinase in the presence of magnesium and Adenosine triphosphate. The glucose-6-phosphate is further oxidized to phosphogluconate with concurrent reduction of Nicotinamide Adenine Dinucleotide (NAD^+) into Nicotinamide Adenine Dinucleotide reduced (NADH). The change in absorbance at 340/380 nm was proportional to the concentration of the random blood sugar in the sample. This concentration was given automatically by the Beckman coulter 480 analyser.

3.12 Sample size calculation

The sample size was calculated based on the comparison of two groups (Eng, 2003). In this method the mean difference of the two groups would be obtained from previous studies conducted and standard deviation from the population. No previous studies had been conducted in Zambia but a similar study had been conducted in Nigeria (Babalola et al., 2007) with mean Selenium of 0.188 mg/L in healthy adults and 0.136 mg/L in essential hypertensive adults with a minimum difference of 0.052. However, for the present study the minimum difference was modified to detect a much smaller difference of 0.02. This was done after taking about 20 measurements from the study population and from which the standard deviation was also determined from to be 0.056.

$$N = \frac{4\sigma^2(Z_{crit} + Z_{pwr})^2}{D^2},$$

Where:

N = the sum of the sizes of both comparison groups

ч = Assumed standard deviation.

Z_{crit} = 1.96 for Ŭ=0.05

Z_{pwr} = 0.842 for statistical power 80%

D = Minimum expected difference between two means.

$$N = \frac{4*0.056^2(1.96 + 0.842)^2}{(0.02)^2}$$

=246.

This meant that an equal number of 123 participants from each group were to be enrolled. However 126 hypertensive and 119 normotensive participants were enrolled. 1 participant was excluded from the study due to renal dysfunction.

3.13 Data collection

The information collected was recorded in the data sheet and finally numerical data entered in STATA version 13 (Stata Corporation, College Station, Texas).

3.14 Variables of interest

Table 3.3 presents the variables of interest and their descriptions.

Table 3.3 Descriptions of variables

Type of Variable	Definition of Variables	Type
Dependent variable Selenium levels	mg/L	Continuous
• Blood pressure (systolic and diastolic	mmHg	Continuous
• Sex	Sex-(Gender) male or Female	Categorical
• Age	Age in years	Continuous
• Weight	Weight in kilograms (Kg)	Continuous
• BMI	Kg/m^2	Continuous
• Urea	mmol/L	Continuous
• Creatinine	umol/L	Continuous
• Sodium	umol/L	Continuous
• Potassium	umol/L	Continuous
• Blood sugar	umol/L	Continuous

3.15 Data analysis

Descriptive statistics: For continuous variables (Selenium levels, age, weight, height, creatinine, urea, potassium and sodium), mean and standard deviation was used for normally distributed data. Percentages were used for categorical variables such as sex.

Analytical statistics*:* Student paired t-test was used to assess the source of variation for continuous variables of age (years), weight (kilograms), height (metres), serum selenium (miligrams per litre), BMI, creatinine (milimole per litre), urea (milimole per litre), sodium and potassium (micromole per Litre); multiple linear regression was used to find relationship of selenium levels with BMI, age and blood pressure using STATA 13.

3.16 Ethical consideration

Before conducting this study the protocol was first submitted to ERES CONVERGE which reviewed and approved the study. Additional approval was obtained from the University Teaching Hospital to conduct the study at the hospital. The information collected from this study was strictly confidential and no names of participants where used but only codes. Participants were assured that information would be disseminated to relevant authorities and with no direct link to them in order to maintain anonymity and that result of this study would be used to improve the health status of the patients affected by essential hypertension. The study participants were recruited based on their willingness to volunteer to take part in the study, they were informed through a consent form about the pain they would experience at the injection site during collection of blood and assurance was given that they would only be handled by well qualified medical personnel.

CHAPTER FOUR: RESULTS

This section shows results obtained after analysis of the data that was collected.

4.1 Characteristics of study Participants

Several demographic and other characteristics of the participants were captured. Among these include: age, gender, weight and height to mention a few. Gender was the only categorical variable and percentages were used to describe the gender distribution. Figure 4.1 below shows the distribution of gender between the two groups i.e. normotensive and essential hypertensive The majority slightly over 50% of the participants in both normotensive (n=60) and essential hypertensive (n=65) cases were female adults and this was compared to males where about 50% (n=59) were normotensive and about 48% (n=61) were hypertensive. Other variables, continuous, are summarised and presented later on.

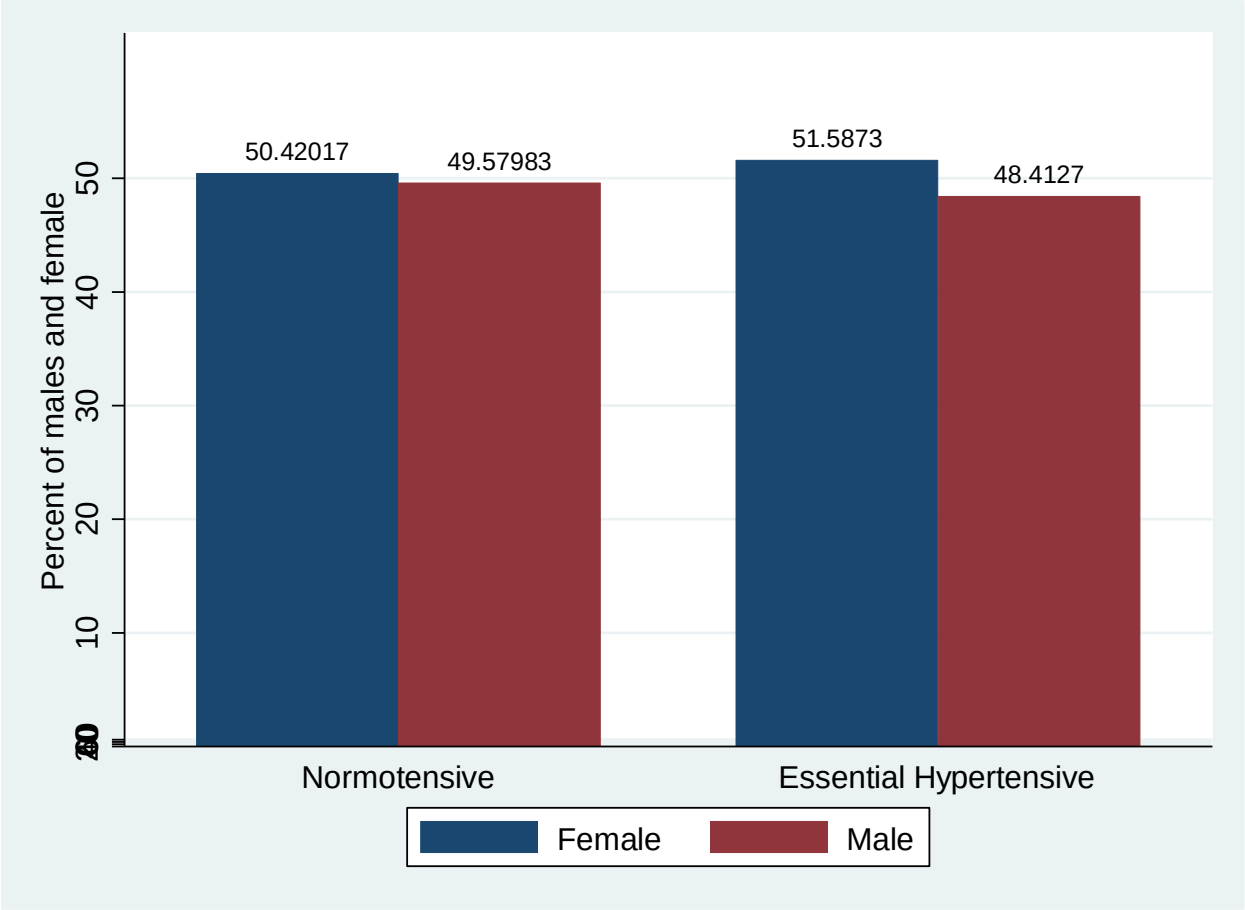

Figure 4.1 Gender percentage distributions by normotensive and essential hypertensive cases.

4.12 Summary Descriptive statistics of study participants for continuous variables

Table 4.1 presents the mean and standard deviation of continuous variables that were normally distributed for normotensive and essential hypertensive adults. There was no significant difference in age between normotensive 47.05 ± 9.96 years and essential hypertensive 48.28 ± 9.60 years (p-value 0.33). The blood pressure for hypertensive participants was significantly higher than in normotensive participants at 5 % level of significance. Similarly, the mean pulse for normotensive participants 70.59 ± 5.36 beats/min was less than those with essential hypertension 75.67 ± 11.52 beats/min and the serum selenium levels in unmatched participants insignificant ($p<0.111$). There was no statistical significant difference in height ($p<0.21$) between normotensive and essential hypertensive. But, there was a difference in weight with those with hypertension weighing more than their counter parts (p-value 0.05). Differences in mean values of creatinine, potassium and sodium levels were not statistically significant as well as that of selenium levels in unmatched participants. Urea, glucose levels and BMI were all significantly higher for those with hypertension compared to the normotensive participants at 5% level of significance.

Table 4.1 Mean serum selenium levels and other key variables (unmatched cases)

Variables	Normotensive (n=119)	SD	Essential Hypertension (n=126)	SD	Difference	(p-value)
Age (years)	47.05	9.96	48.28	9.60	1.23	0.327
Sys-BP (mmHg)	111.64	9.89	158.60	15.06	46.95	*p<0.0001*
Dias-BP (mmHg)	72.908	10.31	96.05	7.92	23.14	*p<0.0001*
Pulse (beats/min)	70.59	5.36	75.67	11.52	5.08	*p<0.0001*
Height (m)	1.66	0.091	1.65	0.068	-0.013	0.207
Weight (kg)	66.622	10.70	69.24	10.53	2.62	0.05
Selenium (mg/L)	0.109	0.047	0.099	0.052	-0.010	0.111
Urea (mmol/L)	3.72	1.024	4.169	0.997	0.451	*p<0.0001*
Creatinine (umol/L)	70.52	9.71	70.31	13.60	-0.214	0.887
Potassium (mmol/L)	4.36	0.551	4.41	0.543	0.046	0.514
Sodium (mmol/L)	139.64	3.17	139.89	3.02	0.249	0.530
Glucose (umol/L)	4.29	0.678	4.57	0.839	0.284	*p<0.0001*
BMI (kg/m^2)	24.04	3.39	25.34	3.28	1.30	*p<0.0001*

*SD ð Standard Deviation

Analysis of matched cases in normotensive and essential hypertension by age and gender

Table 4.2 presents the mean, standard deviation and mean differences of key variables between normotensive and essential hypertensive cases matched by age and gender. There was significant difference in BP levels between normotensive and essential hypertensive cases with a $p<0.0001$. The mean pulse for normotensive participants 70.92 ± 5.43 beats/min was significantly less than those with essential hypertension by about 5 beats/min ($p<0.0001$). The weight was less in the normotensive than essential hypertensive adults but the difference was insignificant ($p<0.07$). Selenium levels were significantly higher in the normotensives than essential hypertensive groups ($p<0.0001$). The mean differences in urea and glucose levels between normotensive and hypertensive participants were significant at 5% respectively.

Table 4.2 Mean serum selenium levels in normotensive and essential hypertensive: matched cases by age and gender

Variables	Normotensive (n=119)	SD	Essential Hypertensive (n=119)	SD	Difference	p-value
Age (years)	46.98	10.00	46.98	10.00	0	1
Sys-BP (mmHg)	111.99	10.07	159.4	10.07	47.37	<0.0001
Dias-BP (mmHg)	72.53	10.19	96.54	10.19	24	<0.0001
Pulse (beats/min)	70.92	5.430	75.80	5.427	4.88	<0.0001
Height (m)	1.67	0.092	1.65	0.092	-0.020	0.080
Weight (kg)	66.37	10.77	69.09	10.77	2.723	0.073
Selenium (mg/L)	0.109	0.047	0.093	0.048	-0.016	<0.0001
Urea (mmol/L)	3.71	1.070	4.09	1.070	0.379	0.008
Creatinine (umol/L)	70.71	9.770	70.38	9.770	-0.329	0.848
Potassium (mmol/L)	4.36	0.550	4.410	0.550	0.051	0.496
Sodium (mmol/L)	139.61	2.86	139.96	2.86	0.36	0.397
Glucose	4.26	0.67	4.52	0.668	0.265	0.010
BMI (kg/m^2)	23.88	3.40	25.42	3.40	1.54	<0.0001

Table 4.3 presents the mean difference in Selenium levels between normotensive and essential hypertensive male participants only. The results show that the mean difference in serum Selenium levels between normotensive 0.115 ± 0.052 mg/L and hypertensive 0.112 ± 0.051 mg/L males only was not significant with a $p<0.7577$. On average, Selenium levels are higher in normotensive males compared to hypertensive ones by 0.003 mg/L.

Table 4.3 Means and mean differences of selenium in normotensive and essential hypertensive males

Group	Observations	Mean	Std. Err.	Std. Dev.	[95% Conf. Interval]	
Normotensive	48	0.115	0.007	0.052	0.100	0.130
Hypertensive	48	0.112	0.007	0.051	0.097	0.127
Mean Difference		0.003				

$p<0.7577$

In contrast, Table 4.4 shows the results for the difference in the mean selenium levels between normotensive and hypertensive females only. The results show that the average selenium level for normotensive females was 0.102 ± 0.042 mg/L while that for the hypertensive ones was 0.0852 ± 0.048 mg/L. The resulting difference of 0.017 mg/L between the two groups was not statistically significant at 5% given $p<0.0632$.

Table 4.4 Means and mean differences of serum selenium in normotensive and essential hypertensive females

Group	Observations	Mean	Std. Err.	Std. Dev.	[95% Conf.	Interval]
Normotensive	53	0.102	0.006	0.043	0.0900613	0.114
Hypertensive	53	0.085	0.007	0.048	0.072	0.098
Mean Difference		0.017				

P<0.0632

4.3 The relationship between serum selenium levels, BMI, age, and blood pressure

To determine the association between serum Selenium levels BMI, age, and blood pressure multivariate linear regression was applied. The dependent variable used in the regression analysis was serum selenium levels. The independent variables included: age, BMI and blood pressure. Figure 4.2 presents the results from the linear regression model. There was no significant relationship among the variables, though we noted a negative relationship between systolic blood pressure and serum selenium levels.

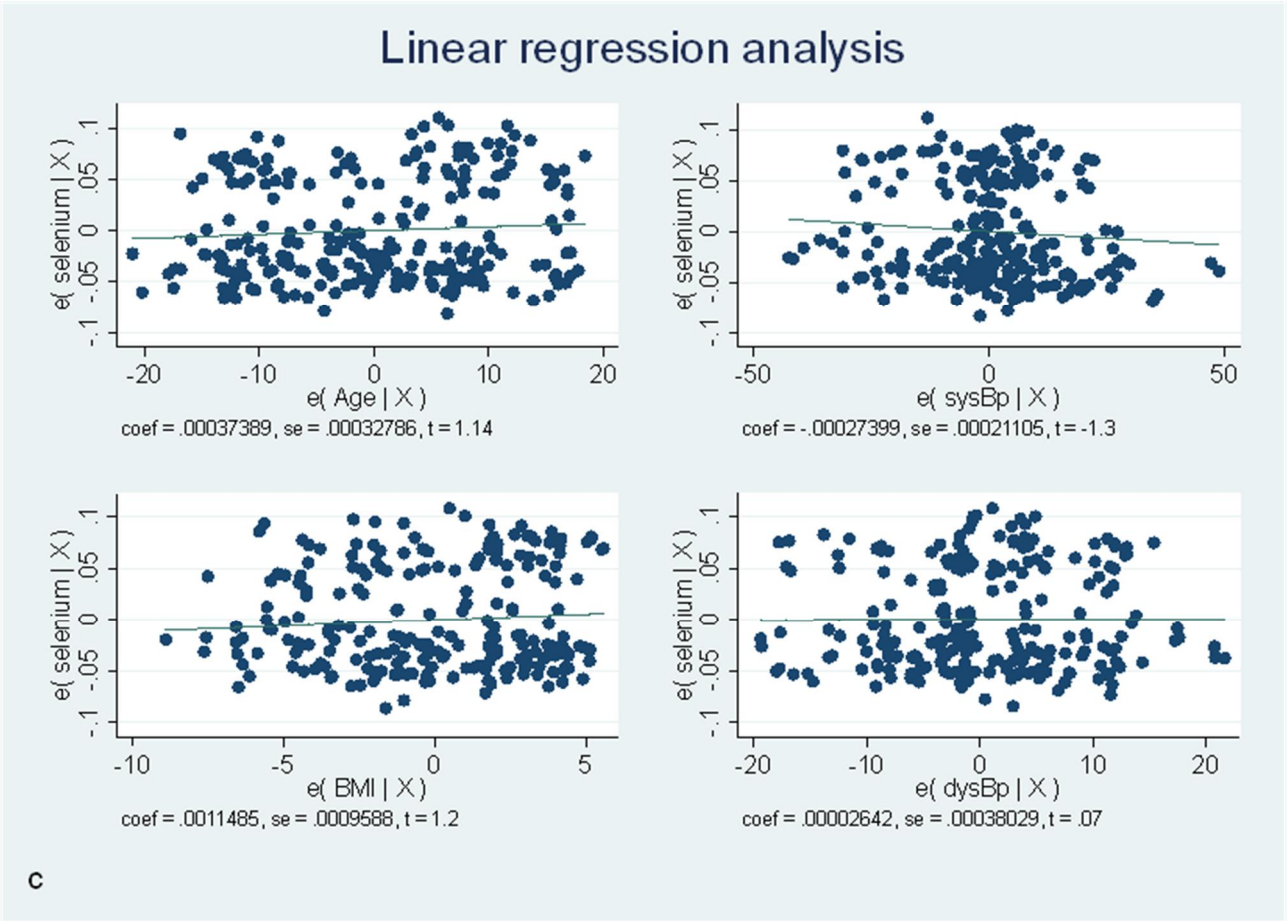

Figure 4.2 Linear regression analysis of serum selenium levels with BMI, age and blood pressure

CHAPTER FIVE: DISCUSSION

5.1 Serum selenium levels in normotensive and essential hypertensive adults

The present cross-sectional study reports a significant difference in serum selenium levels between normotensive (0.109 ± 0.047 mg/L) and essential hypertensive (0.093 ± 0.048 mg/L) participants (p<0.0001). These results are in line with the findings by Babalola et al (2007) who reported lower levels of selenium in hypertension (0.136 ± 0.028 mg/L)) compared to normotensive (0.188 ± 0.026 mg/L). Similarly, Nawrot et al (2007) also reported low levels of selenium in hypertensive adults (0.097 ± 0.019 mg/L) though he did not have comparative group. In addition, da Silva (2017) also reported serum selenium levels to be higher in the normotensive group (0.0564 ± 0.0153 mg/L) than in the hypertensive group (0.0532 ± 0.0152 mg/L) though the difference was not significant and the said study was conducted in pregnant women (da Silva et al., 2017). In contrast, other studies have reported higher levels of selenium in hypertensive adults but with no comparative groups (Laclaustra et al., 2009, Su et al., 2016). The geographical location of an area determines the levels of Selenium in the soil and thus could be used to explain differences in the serum Selenium levels in adults from a given region.

5.2 Serum selenium levels by Gender in normotensives and essential hypertensive adults

Selenium levels in normotensives and essential hypertension were compared by gender too and it was found that there were no significant differences between normotensive females and hypertensive females. These findings were similar to the finding of da Silva et al (2017) though this study was conducted in female pregnant women and had smaller sample size (73 participants). In contrast most studies have found differences in serum selenium levels in women who are normotensive and those with hypertension but most of these studies have been conducted in pregnant women (Haque et al., 2016, Maduray et al., 2017, Ghaemi et al., 2013). This study found no differences between serum selenium levels in normotensive and hypertensive males. Similarly Stranges et al (2011) reported low levels of selenium in essential hypertensive males which were not associated with hypertension. In contrast Nawrot (2007) reported low levels of selenium in males which were associated with hypertension. However, the study design was different from that of the current study and there was no comparison group. Furthermore, study participants were not matched as it was a randomised cross-sectional longitudinal study unlike in this study where participants were matched.

5.3 Selenium levels with BMI, age and blood pressure

There was no significant relationship of selenium levels with BMI (*p*-0.232), age (*p*-0.255) and blood pressure (systolic and diastolic) (*p*-0.195). Similarly other studies did not find any relationship between these variables, though other studies did find a relationship (Sakēz et al., 2016, Santos et al., 2017, Stranges et al., 2011, Letsiou et al., 2014, Agasthi et al., 2015). This current study found no association between essential hypertension and serum Selenium levels in adults. Similar findings to the findings of the present study were reported by studies conducted elsewhere (Christensen et al., 2015, Stranges et al., 2011). In contrast other studies have found a relationship between hypertension and serum selenium levels (Nnodim et al., 2017, Babalola et al., 2007, González et al., 2014,Grotto et al., 2017). BMI can predict serum selenium levels because of the volume of distribution that increase or cause dilutional effect. Shargorodsky et al (2010) reported that combined supplementation of Selenium with other antioxidants lead to lowered blood pressure. The study by Shargorodosky was a randomized controlled placebo study whilst the current study was not which could explain the differences in the findings. The present study had 119 normotensive and 126 essential hypertensive participants matched by age and sex. The study in Nigeria on the other hand had 103 essential hypertensive adults and 88 healthy participants (Babalola et al., 2007).

The present study found no relationship between serum Selenium levels and essential hypertension in adults. The study did however find a negative correlation between serum selenium levels and systolic blood pressure which was statistically insignificant (*p*-0.195). The findings the of current study concurs with many previous studies that found no association of serum selenium levels with essential hypertension (Stranges et al., 2011, Christensen.., 2015, da Silva et al., 2017). Even though no relationship was found between serum selenium levels and blood pressure; the differences in serum selenium levels between normotensives and essential hypertensive need to be investigated further with a much larger sample size.

Finally, the study established that there was a significant difference between serum selenium levels in normotensives and essential hypertensive adults. This finding therefore can be a baseline for other studies especially in pregnant women in Zambia with preeclampsia and other cardiovascular diseases. Also the possibility of Selenium being a risk factor in essential hypertension cannot be excluded especially given the differences in serum Selenium levels between normotensives and essential hypertensive adults that were observed by the current study.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The current study found a significant difference in serum Selenium levels between normotensive adults and essential hypertensive adults attending clinic 5 at UTH.

6.2 Study recommendations

The findings of the present study can be used as baseline information for other studies on the levels of Selenium in cardiovascular diseases. The observed differences in serum selenium levels between normotensives and essential hypertensive adultsô calls for a larger study to be conducted focussing at the role of selenium levels in essential hypertension.

6.3 Limitations of the study

The following were the limitations of the study:

1. Changes in levels of serum Selenium and blood pressure over a period of time was not observed as this was a single point in time study of a cross-sectional study.
2. The present study did not measure other antioxidants nutrients such as vitamin E and C that may have a possibility of a protective role in cardiovascular diseases such as hypertension and also did not measure activity of glutathione peroxidase.
3. The Sample size of the present study was also another limitation in that only 245 study participants were enrolled even though this limitation was addressed by matching of the study participants.

REFERENCES

Afanas' EV, I., 2010. Signaling of reactive oxygen and nitrogen species in Diabetes mellitus. *Oxidative medicine and cellular longevity*, *3*(6), pp.361-373.

Agasthi, P., Aloor, S., Yarlagadda, V., Chenna, A. & Onwuanyi, A. 2015. Abstract P050: Association Between Circulating Selenium Levels and Primary Hypertension: A Meta-analysis. Am Heart Assoc

Al-hamdani, I. A. H. 2010. Estimation of Serum Uric Acid, Urea and Creatinine in Essential Hypertensive Patients. *Tikrit Medical Journal,* 16.

Askari, G., Iraj, B., Salehi-Abargouei, A., Fallah, A. A. & Jafari, T. 2015. The association between serum selenium and gestational diabetes mellitus: A systematic review and meta-analysis. *Journal of Trace Elements in Medicine and Biology,* 29, 195-201.

Babalola, O., Anetor, J. & Adeniyi, F. 2007. Low blood selenium: A probable factor in essential hypertension. *African Journal of Biotechnology,* 6.

Baradaran, A., Nasri, H. & Rafieian-Kopaei, M. 2014. Oxidative stress and hypertension: Possibility of hypertension therapy with antioxidants. *Journal of Research in Medical Sciences : The Official Journal of Isfahan University of Medical Sciences,* 19, 358-367.

Benstoem, C., Goetzenich, A., Kraemer, S., Borosch, S., Manzanares, W., Hardy, G. & STOPPE, C. 2015. Selenium and its supplementation in cardiovascular diseaseð what do we know? *Nutrients,* 7, 3094-3118.

Bolívar, J. J. 2013. Essential hypertension: an approach to its etiology and neurogenic pathophysiology. *International journal of hypertension,* 2013.

Christensen, K., Werner, M. & Malecki, K. 2015. Serum selenium and lipid levels: Associations observed in the National Health and Nutrition Examination Survey (NHANES) 2011ï 2012. *Environmental research,* 140, 76-84.

Ļuparigova, F. & Stafilov, T. (2013) Determination of selenium in human blood serum by electrothermal atomic absorption spectrometry.

Da Silva, A. C., Martins-Costa, S. H., Valério, E. G. & Lopes Ramos, J. G. 2017. Comparison of serum selenium levels among hypertensive and normotensive pregnant women. *Hypertension in pregnancy,* 36, 64-69.

Eng, J. 2003. Sample size estimation: how many individuals should be studied? *Radiology,* 227, 309-13.

Fairweather-Tait, S. J., Bao, Y., Broadley, M. R., Collings, R., Ford, D., Hesketh, J. E. & Hurst, R. 2011. Selenium in human health and disease. *Antioxidants & redox signaling,* 14, 1337-1383.

Fordyce, F. M. 2013. *Selenium deficiency and toxicity in the environment*, Springer.

Ghaemi, S. Z., Forouhari, S., Dabbaghmanesh, M. H., Sayadi, M., Bakhshayeshkaram, M., Vaziri, F. & Tavana, Z. 2013. A prospective study of selenium concentration and risk of preeclampsia in pregnant Iranian women: a nested caseï control study. *Biological trace element research,* 152, 174-179

Giacosa, A., Faliva, M. A., Perna, S., Minoia, C., Ronchi, A. & Rondanelli, M. 2014. Selenium fortification of an Italian rice cultivar via foliar fertilization with sodium selenate and its effects on human serum selenium levels and on erythrocyte glutathione peroxidase activity. *Nutrients,* 6, 1251-1261.

Goma, F. M., Nzala, S. H., Babaniyi, O., Songolo, P., Zyaambo, C., Rudatsikira, E., Siziya, S. & Muula, A. S. 2011. Prevalence of hypertension and its correlates in Lusaka urban district of Zambia: a population based survey. *International Archives of Medicine,* 4, 34-34.

González, J., Valls, N., Brito, R. & Rodrigo, R. 2014. Essential hypertension and oxidative stress: New insights. *World Journal of Cardiology,* 6, 353-366.

Grotto, D., Carneiro, M. F. H., De Castro, M. M., Garcia, S. C. & Junior, F. B. 2017. Long-Term Excessive Selenium Supplementation Induces Hypertension in Rats. *Biological trace element research*, 1-8.

Handy, D.E. andLoscalzo, J. 2016, *Selenoproteins in cardiovascular redox pathology*, in *Selenium*. Springer. p. 463-474.

Haque, M. M., Moghal, M. M. R., Sarwar, M. S., Anonna, S. N., Akter, M., Karmakar, P., AHMED, S., SATTAR, M. & ISLAM, M. S. 2016. Low serum selenium concentration is associated with preeclampsia in pregnant women from Bangladesh. *Journal of Trace Elements in Medicine and Biology,* 33, 21-25.

Kuruppu, D., Hendrie, H. C., Yang, L. & Gao, S. 2014. Selenium levels and hypertension: a systematic review of the literature. *Public Health Nutrition,* 17, 1342-1352.

Laclaustra, M., Navas-Acien, A., Stranges, S., Ordovas, J. M. & Guallar, E. 2009. Serum selenium concentrations and hypertension in the US population. *Circulation. Cardiovascular quality and outcomes,* 2, 369-376.

Letsiou, S., Nomikos, T., Panagiotakos, D., Pergantis, S., Fragopoulou, E., Pitsavos, C., Stefanadis, C. & Antonopoulou, S. 2014. Gender-specific distribution of selenium to serum selenoproteins: Associations with total selenium levels, age, smoking, body mass index, and physical activity. *Biofactors,* 40**,** 524-535.

Maduray, K., Moodley, J., Soobramoney, C., Moodley, R. & Naicker, T. 2017. Elemental analysis of serum and hair from pre-eclamptic South African women. *Journal of Trace Elements in Medicine and Biology*.

Mehdi, Y., Hornick, J.-L., Istasse, L. & Dufrasne, I. 2013. Selenium in the environment, metabolism and involvement in body functions. *Molecules,* 18, 3292-3311.

Melse-Boonstra, A., Hogenkamp, P. & Lungu, O. I. 2007. Mitigating HIV/AIDS in Sub-Saharan Africa through selenium in food.

Mistry, H. D., Broughton Pipkin, F., Redman, C. W. G. & Poston, L. 2012. Selenium in reproductive health. *American Journal of Obstetrics and Gynecology,* 206, 21-30.

Montezano, A. C. & Touyz, R. M. 2012. Molecular Mechanisms of Hypertensionð Reactive Oxygen Species and Antioxidants: A Basic Science Update for the Clinician. *Canadian Journal of Cardiology,* 28, 288-295.

Mulenga, D., Siziya, S., Rudatsikira, E., Mukonka, V., Babaniyi, O. & Songolo, P. 2013. District specific correlates for hypertension in Kaoma and Kasama rural districts of Zambia. *Rural Remote Health,* 13, 2345.

Murray. R.K., Granner. D.K., Mayes. P.A. and Rodwell.V.W. (ed.), 2003. Harper's illustrated biochemistry.26th ed.New York:McGraw-Hill.

Nawrot, T. S., Staessen, J. A., Roels, H. A., Hond, E. D., Thijs, L., Fagard, R. H., Dominiczak, A. F. & Struijker-Boudier, H. A. 2007. *Blood pressure and blood selenium: a cross-sectional and longitudinal population study*.

Nguyen, H., Odelola, O. A., Rangaswami, J. & Amanullah, A. 2013. A review of nutritional factors in hypertension management. *International journal of hypertension,* 2013.

Nnodim, J., Emmanuel, N., Hope, O., Nwadike, C., Ukamaka, E. & Christian, O. 2017. Membrane potential, serum calcium and serum selenium decrease in preeclampsia subjects in Owerri. *Universa Medicina,* 36, 88-93.

World health Organisation. 2015. A global brief on hypertension: silent killer, global public health crisis. *Women*.

Preedy, V. R. 2015. *Selenium : chemistry, analysis, function and effects,* Cambridge, Royal Society of Chemistry.

Rayman, M. P. 2012. Selenium and human health. *The Lancet,* 379, 1256-1268.

Sacco, J. E., Dodd, K. W., Kirkpatrick, S. I. & Tarasuk, V. 2013. Voluntary food fortification in the United States: potential for excessive intakes. *European journal of clinical nutrition,* 67, 592-597.

Sakɐ, D., Kaya, A & Kulaksizoglu, M.2016. Serum Selenium Levels in euthyroid nodular thyroid diseases. Biological trace element research.174, 21-26

Santos, C., García-Fuentes, E., Callejón-Leblic, B., García-Barrera, T., Gómez-Ariza, J. L., Rayman, M. P. & Velasco, I. 2017. Selenium, selenoproteins and selenometabolites in mothers and babies at the time of birth. *British Journal of Nutrition***,** 1-8.

Sena, Laura A. & Chandel, Navdeep S. 2012. Physiological Roles of Mitochondrial Reactive Oxygen Species. *Molecular Cell,* 48, 158-167.

Shargorodsky, M., Debby, O., Matas, Z. & Zimlichman, R. 2010. Effect of long-term treatment with antioxidants (vitamin C, vitamin E, coenzyme Q10 and selenium) on arterial compliance, humoral factors and inflammatory markers in patients with multiple cardiovascular risk factors. *Nutrition & Metabolism,* 7, 55.

Stranges, S., Galletti, F., Farinaro, E., Dôelia, L., Russo, O., Iacone, R., Capasso, C., Carginale, V., De Luca, V., Della Valle, E., Cappuccio, F. P. & Strazzullo, P. 2011. Associations of selenium status with cardiometabolic risk factors: An 8-year follow-up analysis of the Olivetti Heart Study. *Atherosclerosis,* 217, 274-278.

Su, L., Jin, Y., Unverzagt, F. W., Liang, C., Cheng, Y., Hake, A. M., Kuruppu, D., MA, F., Liu, J. & Chen, C. 2016. Longitudinal association between selenium levels and hypertension in a rural elderly Chinese cohort. *The journal of nutrition, health & aging,* 20, 983-988.

Subash, P., Gurumurthy, P., Sarasabharathi, A. & Cherian, K. 2010. Urinary 8-OHdG: a marker of oxidative stress to DNA and total antioxidant status in essential hypertension with South Indian population. *Indian Journal of Clinical Biochemistry,* 25, 127-132.

Teliġman, S., JurasoviĹ, J., Pizent, A. & CvitkoviĹ, P. 2001. Blood Pressure in Relation to Biomarkers of Lead, Cadmium, Copper, Zinc, and Selenium in Men without Occupational Exposure to Metals. *Environmental Research,* 87, 57-68.

Van De Vijver, S., Akinyi, H., Oti, S., Olajide, A., Agyemang, C., Aboderin, I. & KYOBUTUNGI, C. 2014. Status report on hypertension in Africa-Consultative review for the 6th Session of the African Union Conference of Ministers of Health on NCDôs. *Pan African Medical Journal,* 16.

Yan, L., Chi, B., Sindano, N., Bosomprah, S., Stringer, J. & Chilengi, R. 2015. Prevalence of hypertension and its treatment among adults presenting to primary health clinics in rural Zambia: analysis of an observational database. *BMC Public Health,* 15, 933.

ANNEXES

Annex 1 Information sheet

Title of Study; A comparative study of serum selenium levels in normotensive and essential hypertensive adults of 18 to 65 Years old, at the University teaching Hospital, Lusaka, Zambia.

Dear Participant,

This research study is being conducted by Ms Angela Chiti Chisulo a student at the University of Zambia, School of Medicine, under Department of Physiological Sciences. I am informing you of the study as well as requesting you to take part in the study.

There has been a rise in the number of cases of High blood pressure in Zambia and several causes have been associated with this increase. 95% of these of cases of high blood pressure are as a result of unknown causes. This is referred to as essential or primary hypertension. In view of this, there is a need for efforts to be made to overcome this problem. This study aims at finding out if there are differences between serum selenium levels in people with high blood pressure and those without high blood pressure. This trace metal is obtained mostly from diet and is essential as it forms part of antioxidant enzymes that help in overcoming oxidative stress which is one of the risk factors in developing high blood pressure.

Firstly, a physical examination will be carried out to determine your health status by your physicians as part of their standard care of patients. This physical examination which is in form of a questionnaire requires you to give information on your age, sex (gender), past medical history, family history, and common symptoms, including examining your heart, chest, legs, arms, muscles and your general condition. With your permission this information will be used in the study.

After this, 8mls of blood and 10 mls of urine will be collected from you by a nurse. Blood and urine samples will only be collected once.

Physical risks of pain and discomfort when collecting blood may be encountered but you should not worry as you will be attended to by qualified Nurses. Some physical risks include dizziness, sweating, coldness of skin, numbness and tingling of feet, nausea, vomiting, possible visual disturbance, swelling around the area where the needle was injected. If you get any of these

signs please immediately inform your doctor or the nurse attending to you so that you are immediately treated.

You will not get any direct or monetary gain by taking part in this research as your participation in this study is voluntary. If you are not interested in this study you are eligible to withdraw without any punitive measures against you or affecting your acquisition of health services. The blood and urine samples will be used to gather information which will help in establishing the association between serum selenium levels and High blood pressure. All the information you will provide from the physical examination as well as that from the blood and urine tests will strictly confidential and not linked to you. Please seek clarification where you do not understand.

PERSONS TO CONTACT FOR PROBLEMS

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Annex 2 Informed consent form

The reason for this study has been explained to me clearly and I understand the aim, risk and benefits as well as confidentiality of taking part in the study. I also understand that if I agree to take part in the study. I can withdraw without giving an explanation for my doing so.

I have been informed of the following:

1. The amount of blood to be takené (Specify the amount).

2. The amount of urine to be donatedé .. (Specify amount).

3. The number of times of blood and urine donationsé é é é é é é é é é é é .....

(Specify the number of times).

4. I understand the use that will be made of the samplesé é é é Indicate YES OR NO

I é . (Names)

Agree to take part in this study

Signature é é é é é é é é é é é .date ;é é é é é é é é é é é é (Participant)

Participantôs signature or thumb print

Name of the interviewer; é

Signature; é é é é é é é é é é é date; é é é é é é é é é é é é é é é é é é é .

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Icishibisho che sambililo ili (Information sheet in BEMBA)

Umutwe we sambililo: isalambililo lya kwishiba pali selenium muba lwele ba BP nabo aba shalwala ubu bulwele, abali nemyaka óyakufyalwa ukwambila pa 18 ukufika na 65. Ili sambililo, likacitikila pa cipatala cikalamba ica University Teaching hospital,Lusaka,Zambia.

Kuli imwe ba kacele wandi,

Ulu lubali lwe sambililo lulecitwa naba Angela Chiti Chisulo (Ms) abali kumasambililo ya pamulu pe sukulu likalamba iyitwa University of Zambia, pe sukulu ya kusambilila ubumi bwa bantu.Ndemishibisha pali isambilili nokumilomba ukutila ati mwengasenadamo ulibali muli ili sambililo.

Ici cisambililo cikalolekesha pa bulwele bwa BP. Ubu ubulwele bulekulilakofye inshita ne nshita muno mu Zambia. Kwaliba ifintu ifingi ifingalenga ubu bulwele ukufula mu bantu. Impendwa ikalamba iya ubu bulwele bwa BP bu lengwa ne fintu ifi shaishibikwa. Eicho, amaka yafwile ukubikwako pakuti twingacinfye ubu bwafya. Ili sambililo lileyesha ukwishiba impendwa selenium mu mulopa mu ba lwala BP namuli abo abashalwala BP kutiyalenga ukukwata ububulwele.

Icakwambilapo, ukucecetwa kwa ku mubili kwalacitwa na ba shingaanga pamo ngefyo babomba cilanshiku. Uku kucecetwa, kwesha ukumona nga muli abomi abo abashinga kwata amalwele ayasenda inshita ntali ukuti yapole. Umu mukucecetwa, mule fwaikwa ukupela imyaka mukwete, mufwile ukulanda ngamuli bana kashi olo abaume, mufwile ukulanda amalwele ayo mwalwalapo, namalwele yamulupwa, kabili mufwile mwalondolola ifyo mumfwa limo-limo, nangu ngakwaliba ifimicusha mumubili wenu, ba shingaanga balayesha kabili no kumiceceta konse-konse, ukwambila kumutima, amolu, iminofu nemimonekele ya mubili wenu,Ngamwasuminisha tukabomfyako lusebo mwisambililo lyesu .

Panuma yaku cecetwa, umulopa ukulingana nama mls ayali yane (8mls) eyalasendwa ukufuma kuli baimwe, ukulundapofye, mwalafwaikwa ukupelako nemisu mukakunkubiti akanono eko balamipela ulingene na 8 ml olo ukupitilila. Ukukupela kwaifi fintu kwalafwaikwa ukucitwafye umukumo mpo fye. Umulopa ne misu mwala tupela fikabomba ngefi: kumulopa; tukesha ukwishiba ubwingi nangula ukucepa kwa selenium. Imisu nasho shikabomba mukusanga ifintu ifingalufyana ubumi bwamuntu.

Ifi, efintu fimo ifingamicitila ilyo mule pela umulopa; Kuti mwaiumfwa ulushingwa,umuselu,nokufimba pa cende apo bafumishe umulopa kabili nokukalipa. Mukwesha

ukucefya amasanso aya; abakalasende uyu mulopa niba nurse abaishiba bwino iyi nchito kabili nshindano ishisha bombapo eshikaba ishakubomfeshiwa.

Ishibeni ukutila, takwakabe ukupelwa ka museke panuma yakusendamo ulubali mulici cisambililo. Ukulolesha pa mulopa ku kalunda ubwishibilo bwakwafwa abo balecushiwa no bu lwele bwa BP. Ukusendamo ulubali muli cicisambililo kwena kuli fye kusala kwenu, emulandu wine ngamwaisafwaya ukufumamo, tapali umuntu uwukamupatikisha ukutwalilila. Elyo, ukundapishiwa kwenu pacipatala Ăci, takwacilinganishiwe nakalya awe.Apo tamumfwikishe, ipusheni.Amaasuko yonse eyo mwakulapela, yena yalaba ninkama iyo iyitayakasokoloke kumuntufye nelyo umo. Lelo panuma yaici cisambililo, ulusebo lukatwalwa ku ma office yakalamba, ayo ayashakeshibe amashina yenu. Lelo bakabomfya ulu lusebo ukumona nga icisambililo icikalamba kuti cacitwa mukwebati be ngafwa abekala calo abali nobu bulwele bwa BP.

ABANTU BAKUMONA NGAMWASANGWA NAMAFYA PAMBALI YAKUITWALA KWI SAMBILILO ILI.

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Icakulangililako ukwebati ni nsumina ukaba muli ili sambililo (Concent form in BEMBA)

Ubukulu bwa ili sambililo na bulondololwa kuli ine, ningufwikisha epo ili sambilo li shintilile no bwafo bose ebo ndesendamo, na masanso yonse ayengasangwamo, ne misungile yankama muli ili sambililo. Kabili ni ngufwikisha ukutila; nganasumina ukuba palyabo muli ili sambililo, kuti na fumumo ukwabula ukupela ubulondoloshi ku muntu uuli onse, pantu ukuba muli ili sambililo kulefuma pa busalo bwandi ubo mpangile.

Ineé .nasumina ukupela umulopa na Ămisu ukubomfeshewa naba é é é é é é é é é é é é é é é é ...

Nabanjishibisha palifi:

1. Ubwingi bwamulopa ndepeelaé é é é é é é é é é é é é é é é é é é (lembeni ubwingi).

2. Ubwingi bwamisu ndepeelaé é é é é é é é é é é é é é é é (lembeni ubwingi).

3. Imiku ya kupeela imisu nomulopaé é é é é é é é é é é é .....

(lembeni imiku).

4. Incito iyo umulopa na misu iikabombaé é é é é lembeni emukwai nangu aweh

Na sumina ukuti imisu na ómulopa kuti fya sunga no kubofibomfya mu nshiku ishileisa emukwai/awe mukwai

Icishibilo ca ba kapelaé é é é é é é é é é é é é é é é ubushikué é é é é ..

Icishibilo ca ba kasenda wa mulopaé é é é é é é é é é ...ubushikué é é é ..

A. ABANTU BAKUMONA NGAMWASANGWA NAMAFYA PAMBALI YAKUITWALA KWI SAMBILILO ILI.

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Uthenga wofotokoza za kufufuza kukuchitidwa (Information sheet in CHINYANJA)

Mutu wa Zofufuzidwa: Kupalanisa kwa kuchepa olo kupaka kwa selenium mugulu la antu ali na matende othamangitsa mutima na aja ilibe matenda aya alina zaka zakubadwa 18 ndi 65 muchipatala chachikulu University Teaching Hospital, Lusaka, Zambia.

Kwa Otengako mbali mu kufufuza uku,

Uku kufufuza kudzachitidwa ndi ba Angela Chiti Chisulo.amene ali pa maphunziro apamwamba pa sukulu yaikulu (University of Zambia), kupunzila za mankwala ndi kuchilitsa, kumbali ya mayendedwe a zinthu muthupi nakumipempani kuti mu. Ndikuziwisani za kufufuza uku nakumi pempani kuti mutengenko mbali mu kufufuza uku.

Kufufuza uku kudzankala pa matenda Othamangitsa mutima omwe aliku kulilako mu dziko la Zabiya (Zambia).Matenda aya yalengatsewa na zintu zo pusa pusana.Koma nthawi yambili zolenga matenda Othamangitsa mutima sivizibika. Nichifukwa cha ichi chameni tifunika kufakilako mphamvu kuti tichinfye aya matenda.Uku kufufuza kuyesa kuona ngati kupaka olo kuchepa kwa selenium mu mulopa mwa antu alina BP an anja alibe BP ku ngalenge muntu kun kala namatenda BP.

Choyambilila, a kastwili, a sing'anga (Dotolo/dokotala) olembesedwa ku boma omwe a sebenza nchito iyi masiku onse adza pima kuti adziwe zatanzi lanu panthawiyo comanso nthawi zakumbuyo. Muyenele ku a dziwisa; zaka zakubadwa, kapena ndunu amuna olo akazi), azafutsanso kuti aziwe za matenda amene ali mubanja lanu.Azaona zizindikilo za matenda pa thupi panu ndipo azapina kasebenzedwe ka mutima, muchfuba; miyendo, manja nthanze ndiponso zina ndi zina zokhuza thanzi lanu. Mukavomeretsa tizasebenzesako Uthenga Uyu mu kufufuza kwatu.

Akatha kupima ndikufunsa zonese. Anurse azatenga magazi okwanila 8 mls na mikozo yonso izafunika kutengedwa muka container yolingana 8 mls olo kupitilila. Zomwe azapima mu magazi ndi mikodzo yonse idzatengedwa zili motele:Adzapima selenium.Mu mikozo aza pima ma electrolytes, creatinine, and urea. Mikozo iza pimidwe mwanthawizonse ndi topimila to chedwa strips.

Zodetsa nkhawa pa kupeleka magazi ndi izi: mwina, atatenga magazi, yena maga zi yanga khale kunsi kwa chikumba busi nu thimbilila pa malo awa. Mwina panga vimbe.Anthu ena amanvva chizwezwe, mwina nu piba, bena amanva ka mphepo, nzanzi, museluselu, mwinanso uchita chidima pa maso, bena banthu anga komoke. Ofufuza adziwa za izi ndipo ayesa kuika mumalo

zina ndi zina zoyetsetsa kuti zododometsazi zitsachitike. Adziwitse mwamsanga a dotolo kapena a tsinganga akaona zizindikilo zo sonyeza chilichonce mwa zododomesazi.

Odzipeleka akupemphedwa kudipeleka mwa ulele. Kulibe ndalama yomwe mudzapatsidwa kapena mphoto ina yanu yanu chifukwa chotengako mbali mukufufuza uku. Ngati mwadodoma, muli omasuka kukana kutengako mbali mu kufufuza uku. Mukakana kutengako ku mbali iye kulibe zi za mitchitikila.Adotolo amu Chipatala azamiuonani na mankwanla mutzatenga mu Chipatala.Uthenga ndi mayankho omwe mudzapatsa pa kufufuza uku, kudzasungidwa mu chinsinsi. Zopeza pa kufufuzaku zizatumidzidwa kumaofesi akulu muchiisinsi chamaina komanso zonse zina zowadziwitsa za otengako mbali.Ngati zinna zolembedwa mu ndime izi sizinanveke, funsani.

ANTHU MUNGAUZE NGATI MWAPEZA MAVUTO NDI KUZIPELEKA KAPENA KUTENGAKUMBALI PA KUFUFUZA UKO

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Yovomeleza (Concent form in CHINYANJA)

Andifotokozela nidipo ndavetsetsa cholinga cha kufufuza kuli kuchitidwa, phindu yache, zododometsa zache kapena zogwampwawi zache; comanso chisinsi cha ofufuza pa maina ya anthu otengako mbali pa kufufuza uku.

Ineé . ndivomela kupeleka magazi ndi mikozoé .

Andi fotokozela, ndipo ndavetsa zonse zolembedwa mu mitu ya ndime yotsatilai:

1. Kuchuluka kwake kapena muyeso wa magazi azatengedwa kukapimaé é é é é é é é é é é é é é é é é é é (lembani muyeso).

2. Muyeso wa mikozo womwe muzaelekaé .. (lembani muyeso).

3. Afotokoza ma uledo (mo penda masiku) omwe magazi ndi mikozo izafunika kupelekedwaé é é é é é é é é é é ....(lembani kangati).

4. A fotocoza zomwe azachita ndi magazi komanso mikodzo omwe udzatengedwaé é é é é é é Lenmbani Inde olo Ai

Ndavomekze kuti angasunge magazi ndi mikozo yotengedwayo

Chizindikilo cha odzipeleka (wotengako mbali)é é é é é é é é é é é é é é .

Tsiku la a Chakaé é é /é é é é é /20é é é

Chizindikilo cha otenga magazi bloodé é é é é é é é é é .é é é é é ...

Tsiku la a Chakaé é é /é é é é é /20é é é

ANTHU MUNGAUZE NGATI MWAPEZA MAVUTO NDI KUZIPELEKA KAPENA KUTENGAKUMBALI PA KUFUFUZA UKO

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Annex 3 Physical examination form

ID Number é

Sexé é é ..é é

Ageé é

Medical History

Hypertensioné é

Diabetes é

Renal diseaseé .....

Other chronic diseases (Cushings,Conns syndrome, Pheomochromocytoma)é é é é

Drug historyé é

Family History of Hypertension, Diabetes mellitus, Asthma and Renal disease

é é

Presence of Common Symptoms

	Present	Absent
Headaches	é é ..	é é ..
Chest pains	..é é	é é ..
Abdominal Pains	.é é	é é ..
Easy fatigability	..é é	..é é
Lack of appetite	..é é	..é é
Problems with Vision	é é é	é é .
Bleeding from the nose	é é ..	.

Limbs and General body Swellings (if present specify body part) .....é ...

General Examinations

Blood Pressureé é

Pulseé é

Pedal oedemaé é

Palloré é

Heighté é

Weighté .

Examinations of the respiratory and cardiovascular system

	Normal	Abnormal
Inspection	é é é	é é é
Palpitation	é é é	é é é
Percussion	é é é	é é é
Auscultation	é é é	é é é

If abnormal explainé é

é é

Examination of the central nervous System

	Normal	Abnormal
Reflexes	é é é	...é é é .
Power in extremities	é é é	é é é é

If abnormal explainé ..

é ..

Examination of Abdomen

Yes/No

Any organomegally é é é

If yes explainé .

é .

Musculoskeletal System Examination

Yes/No

Any abnormality é é é é

If yes explainé é

é ..

Laboratory Investigations

Urinalysis

Proteins Glucose ketones Blood Nitrites PH Leukocytes Urobilinogen Bilirubin

Positive é é .. é é é é .é é é é . é é . é é é é . é é é é é é

Negative é é é é é .é é é é .é é é é ... é é ..é é . ...é é .. é é é

Blood samples investigations

Creatinineé é

Random blood sugaré é

Urea and Electrolytesé é é é ..é .