**THE UNIVERSITY OF ZAMBIA**

**SCHOOL OF MEDICINE**

**DEPARTMENT OF PHYSIOLOGICAL SCIENCES**

# A Comparative Study of Prevalence of Glucose-6-Phosphate Dehydrogenase Deficiency in Essential Hypertensive and Normotensive Adults Aged between 35 and 65 years at the University Teaching Hospital, Lusaka, Zambia.

**A Dissertation submitted**
**By**

**DR MWENYA C. KWANGU, BVM**

**In partial fulfillment of the requirements for the award of the Degree of Master of Science in Biochemistry.**

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## CERTIFICATE OF APPROVAL

The University of Zambia approves this dissertation of Dr Mwenya C.Kwangu, in partial fulfilment for the requirements for the award of the degree of Master of Science in Biochemistry.

Examiner's signature Date

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**Examiner I**

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**Examiner II**

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**Examiner III**

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## CERTIFICATE OF COMPLETION OF DISSERTATION

I, **Dr Mwenya C. Kwangu**, do hereby certify that this dissertation is the product of my own work and in submitting it for my Master of Science in Biochemistry programme, further attest that it has not been submitted to another University in part or whole for the award of any programme.

Signature..... Date.....

I, **Dr G.Sijumbila**, having supervised and read this dissertation is satisfied that this is the original work of the author under whose name it is being presented. I confirm that the work has been completed satisfactorily and is ready for presentation to the examiners.

Supervisor: **Dr G.Sijumbila**

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## DECLARATION

This dissertation is the original work of **DR MWENYA C. KWANGU.** It has been done in accordance with the guidelines for MSc. Biochemistry dissertations for the University of Zambia. It has not been submitted elsewhere for a degree at this or another University.

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I have read this dissertation and approved it for final submission.

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## ABSTRACT

### BACKGROUND

Glucose-6-phosphate dehydrogenase (G6PD) enzyme deficiency is the most common enzymopathy in humans and affects over 400 million people worldwide, majority being in Africa. G6PD catalyses the first reaction of the pentose phosphate pathway where it plays a role in generation of NADPH, which is essential in reducing glutathione. In the reduced state, glutathione decreases oxidative stress. Oxidative stress has been shown to adversely affect vasodilation of small vessels which is essential for reducing blood pressure. There is however, limited scientific evidence that associates G6PD deficiency and aetiology of essential hypertension. This study aims at determining the prevalence of G6PD deficiency in both the essential hypertensive and normotensive adults, and also establishing its association to essential hypertension. It will also determine the levels of nitric oxide (NO) in both groups and its association to essential hypertension.

**METHODS**: An analytical cross-sectional design was applied to 89 essential hypertensive participants and 89 healthy normotensive participants, making a total of 178. All the participants were aged between 35 and 65 years. Blood was collected for G6PD activity and serum levels of NO, glucose, creatinine; and, urea and electrolytes. In addition, routine urinalysis was done. A logistic regression was used to investigate the association of age, sex, nitric oxide levels, and G6PD deficiency with essential hypertension as the dependant variable.

**RESULTS**: The G6PD deficiency was found in 14 (16%) participants with essential hypertension and 9 (10%) control participants. The difference however, in the G6PD deficiency prevalence rate was not statistically significant (*p= 0.13*). Logistic regression analysis, including G6PD deficiency, age, nitric oxide, and gender as covariates, revealed that G6PD deficiency was significantly associated with increased risk for essential hypertension (odds ratio [OR]=2.9, 95% confidence interval [CI]=1.07-7.94, *p=0.036*), while nitric oxide was significantly associated, but with reduced risk for essential hypertension (odds ratio [OR]=0.99, 95% confidence interval [CI]=0.98-0.99, *p=0.001*). The analysis further showed a significant association of age and essential hypertension, with participants in age groups 46-55 and 56-65 being at higher risk of developing essential hypertension than those in age group 35-45 (odds ratio for age groups [OR]=2.4, 95% confidence interval [CI]=1.05-5.54, *p=0.037* and [OR]=7.3, [CI]=3.22-16.61,

*p=0.000* , respectively). The study also revealed no association between gender and essential hypertension ([OR]=0.79, [CI]=0.39-1.60, *p=0.52*).

**CONCLUSION**: The study established that the difference in the G6PD deficiency prevalence rates was not statistically significant at 0.05 significance level (*p=0.13*). Therefore, the results support the hypothesis that states that there is no difference in G6PD deficiency prevalence between the essential hypertensive and normotensive adults, though the study showed a possible role of G6PD deficiency in the pathophysiology of essential hypertension. The findings also demonstrated an association between age, nitric oxide levels and essential hypertension.

## DEDICATION

This dissertation is dedicated to my parents; Mr and Mrs Kwangu for their understanding, love and support, my fiancé Chalwe Sunga for her sacrifice and love, and young brothers and sister; Mwamba, Mulenga and Bwalya respectively for their encouragements during my period of study.

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## LIST OF ACRONYMS

| | | |
|---|---|---|
| **ACD** | - | Acid-citrate-dextrose |
| **BC** | - | Bathocuproine |
| **BP** | - | Blood pressure |
| **DNA** | - | Deoxyribonucleic acid |
| **EDTA** | - | Ethylenediaminetetraacetate |
| **G6PD** | - | Glucose-6-Phosphate Dehydrogenase |
| **GSH** | - | Reduced form of Glutathione |
| **GSSG** | - | Oxidised form of Glutathione |
| **NADP** | - | Nicotinamide dinucleotide phosphate |
| **NO** | - | Nitric oxide |
| **RBCs** | - | Red blood Cells |
| **ROS** | - | Reactive oxygen species |
| **TCF** | - | Temperature correction factor |
| **TNBT** | - | Tetra nitro blue tetrazolium |
| **UTH** | - | University Teaching Hospital |
| **WHO** | - | World Health Organisation |

## DEFINITION OF KEY OPERATIONAL TERMS

| | | |
|---|---|---|
| **Essential hypertension** | - | An elevated systemic arterial blood pressure for which no cause can be found |
| **G6PD deficiency** | - | Is a hereditary abnormality in the activity of an erythrocyte (red blood cell) enzyme |
| **Hypertensives** | - | Participants in the study with blood pressure above 140mmHg (systolic) and 90mmHg (diastolic) |
| **Normotensives** | - | Participants in the study without high blood pressure |
| **Metahemoglobin reductase** | - | An enzyme which prevents iron of the haemoglobin from being oxidised |

# Chapter 1

## 1.1 INTRODUCTION

Gucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common enzyme deficiency in humans and affects over 400 million people worldwide. This accounts for 7.5% of the carriers, Africa having 35% and about 0.1% in Japan and some European countries. This makes it the most common clinically significant enzyme deficiency in the world (Castro et al., 2006). Glucose-6-phosphate dehydrogenase (G6PD), catalyses the first reaction of the pentose phosphate pathway. This reaction necessitates the recycling of glutathione from the oxidised (GSSG) to the reduced form (GSH). This is due to the conversion of nicotinamide adenine dinucleotide phosphate ($NADP^+$) to NADPH + $H^+$, which helps to reduce glutathione through the enzyme called glutathione reductase (Efferth et al., 2006).

G6PD deficiency is caused by diverse array of mutations in the *g6pdx* gene, which is X linked and mostly found in males. Common mutant gene variants produce a defective enzyme which is rapidly degraded, resulting in decreased G6PD amounts and its overall enzymatic activity. The World Health Organisation (WHO) has divided the deficiency in varying degrees of severity; class I is very severe deficiency (less than 1% of normal G6PD activity), class II is severe deficiency (1-10% of normal activity), class III is moderate deficiency (10-60% of normal activity), class IV is normal activity 60-150% activity, and class V is increased activity and this accounts for more than 150% of normal activity of G6PD (Ho et al., 2007).

The most deficient alleles (A-and Med) result in moderate deficiency (class III). G6PD A-is largely found in African populations and results in a residue G6PD activity of approximately 20%. The variant A is found in nearly 11% North American black population. However, its frequency is greater in the Sub-Saharan black population. The enzymatic activity of this variant corresponds to the range between 5% and 15% of the normal one. This decrease is due to some amino acid substitutions at various positions. One of these substitutions is similar to the one that appears in variant A+ and the other one, unique for this variant, involves the substitution of valine by methionine in position 68 (Castro et al., 2006; Efferth et al., 2006; Ho et al., 2007).

The importance of G6PD is found in the transcendence of cellular processes in which it takes part. On one hand, NADPH that is produced in the first and the third reactions of the pentose phosphate pathway is used in the biosynthesis of cholesterol and fatty acids and also in the synthesis of nitric oxide (NO). On the other hand it is needed for the activity of metahemoglobin

reductase which prevents iron of the haemoglobin from being oxidised, and for the maintenance of the reduced level of glutathione (GSH). NADPH and GSH are responsible for the reducing potential, which protects red blood cells from the oxidative stress, and maintains the cysteine residues of the cellular membrane proteins, as well as other enzymes in reduced state ideal for the erythrocyte survival (Gaskin et al., 2001).

The G6PD enzyme deficiency may as well be one of the factors that contribute to essential hypertension due to the role it plays in the reduction of oxidative stress. Arterial hypertension is defined as a medical condition characterized by a persistent elevation of blood pressure over 140 mmHg for the systolic and over 90 mmHg for the diastolic pressure (Tepel, 2003). The term "essential hypertension" refers to those cases of arterial hypertension (95%) whose aetiology or origin is unknown, in contrast to those of secondary hypertension where causes are known (5%). Essential hypertension is believed to be caused by "heterogeneous genetic and environmental causes, which despite relentless research efforts, remain incompletely understood" (Tepel, 2003). It is present in 25-35% of the adult population and in 60-70% of people older than 70, and it is the major cause of coronary heart disease and stroke.

In Zambia, the prevalence rate of hypertension is 34.8% (Goma et al., 2011). However, the prevalence has been on the rise in the recent past, a situation that calls for quick interventions.

Attempts to counteract the hypertensive effects of reactive oxygen species (ROS) have led to the use of exogenous antioxidants thought to improve the vascular function and reduce the blood pressure in animal models and human hypertension. Nevertheless, the data available is not conclusive and the relationship between blood pressure and oxidative stress in humans remains to be elucidated (Ho et al., 2000).

From the above discussion, it can be seen that reduced G6PD activity will have an effect on the production of NADPH which may later affect re-cycling of glutathione and synthesis of NO, actions which may culminate into essential hypertension (Gupte et al., 2007).

This study sought to determine the prevalence of glucose-6-phosphate dehydrogenase deficiency in essential hypertensive and normotensive adults, and the relationship of this deficiency to essential hypertension. The study also determined the relationship between nitric oxide levels and essential hypertension.

## 1.2 STATEMENT OF THE PROBLEM

In Zambia the prevalence rate of hypertension is 34.8%. In the recent past, there has been an increase in the levels of hypertension (Goma et al., 2011). Essential hypertension is known to account for 95% of all hypertensive cases compared to 5% due to the secondary causes of hypertension (Tepel, 2003). It is present in 25-35% of the adult population and in 60-70% of people older than 70. It is the major cause of coronary heart disease and stroke.

One of the contributory factors to essential hypertension is oxidative stress which is as a result of the imbalance between the ROS and the antioxidant molecules; a process G6PD deficiency significantly contributes to (Rodrigo et al, 2001). The prevalence rates of G6PD deficiency in the Southern African region where Zambia falls have been documented to be from 15 to 26% (Cappellini and Fiorelli, 2008). However, the prevalence rates in Zambia are not documented; therefore, we studied the G6PD deficiency due to the high prevalence rates in Southern Africa, and the possible significant role this deficiency plays in the development of essential hypertension which is on the rise in Zambia.

## 1.3 JUSTIFICATION

The results of this study will act as baseline for bigger studies regarding the G6PD deficiency. The information gathered will also be useful for policy makers regarding the management of essential hypertension. This may be done after a larger study is carried out. The same information generated from this study, will be useful to other researchers in other areas. For example, in diabetes mellitus, haemolytic anaemia, in malaria where G6PD deficiency offers protection from malarial parasite, and in neonatal jaundice.

## 1.4 GENERAL OBJECTIVE

To determine the prevalence of G6PD deficiency, in essential hypertensive and normotensive adults, at the University Teaching Hospital (UTH).

## 1.5 SPECIFIC OBJECTIVES

1. To determine the difference in the prevalence of the G6PD deficiency in hypertensive and normotensive adults.
2. To determine the relationship between G6PD deficiency and essential hypertension.
3. To establish the relationship between serum NO levels and essential hypertension.

## 1.6 HYPOTHESES

***Null hypothesis:*** There is no difference in G6PD deficiency prevalence between the essential hypertensive and normotensive adults.

***Alternative hypothesis:*** There is a difference in G6PD deficiency prevalence between essential hypertensives and normotensives.

***Note*:** The level of significance will be determined by using the *p*-value of 0.05 (5%); therefore, the Null hypothesis will be rejected and the alternative hypothesis accepted when the *p*-value is less than 0.05, and it will be accepted and the alternative rejected when the *p*-value is above 0.05.

# Chapter 2

## 2.1 LITERATURE REVIEW

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is mainly defined as reduced or decreased activity of the enzyme G6PD, as opposed to complete absence of the enzyme (Luzzatto, 2012). This point is supported by other studies that have determined that the deficiency is as a result of the diverse array of mutation in the gene *g6pd* (Cappellini and Fiorelli, 2008). To emphasise this point, Castro et al, reported in a study of G6PD deficiency prevalence of 2799 new-borns that 39 (1.4%) exhibited total deficiency. This finding raised a possibility of finding total inactivity of this enzyme in populations (Castro et al., 2006). In the present study, the G6PD deficiency was any G6PD activity less than 10.01 U/g Hb.

G6PD deficiency is mainly caused by mutations in the sex linked chromosomes. It is an X-linked genetic condition which is more likely to affect more males than females. The X-linkage has also been demonstrated in animal studies such as mice (Cappellini and Fiorelli, 2008). The gene is polymorphic in populations in which males have only one allele (hemizygous) and females have two G6PD alleles. Females will only have the disease when there are two defective copies of the gene in the genome. Males are hemizygous for the G6PD gene and can, therefore, have normal gene expression or be G6PD deficient. Females, who have two copies of G6PD gene on each X chromosome, can have normal gene expression or be heterozygous (Perez-Crespo et al., 2005). From this, it can be seen that this G6PD deficiency is mostly common in men although this does not tell us that women cannot have it. In this study, both men and women were looked at in order to determine whether there was significant effect of this deficiency to essential hypertension in both male and female.

G6PD deficiency is known to be the most common enzymopathy that affects about 400 million worldwide. The highest prevalence has been reported in Africa, Southern Europe, the Middle East, Southeast Asia, and the Central and Southern Pacific Islands (Cappellini and Fiorelli, 2008). In the study by Cappellini et al, the prevalence of G6PD deficiency in Southern Africa was estimated to be between 15-26%. Although this prevalence includes Zambia, there is paucity of data on the G6PD deficiency prevalence in this country. Luzatto reported in his study that G6PD deficiency in Nigerians ranges from 4% to 26% with the male population having 20-26% (Luzzatto, 1995). In another study conducted by Egesie where 126 male Nigerians were evaluated, it was discovered that the prevalence of G6PD deficiency stood at 20% (Egesie et al., 2008). This prevalence is in line with the earlier stated prevalence rates (20-26%) of G6PD

deficiency prevalence among men. The observed prevalences could be explained by G6PD deficiency being an X-linked condition; hence it is mostly found in males than in females. This is supported by other authors. Abolghasemi reported in his study of 2000 male and female at-term neonates that 2.1% were G6PD deficient (3.6% were males and 0.6% were females) (Abolghasemi et al., 2004). Carvalho also reported in a case control study of 490 new born babies admitted to hospital de clinicas de Porto Alegre for phototherapy, that, there was 4.6% G6PD deficiency prevalence with a boy-girl ratio of 3:1 in jaundiced new-borns. This again shows that this condition is mostly found in males (Carvalho et al., 2011). In a study by Chinevere, where he studied data from 63,302 people, 2.5% of the males and 1.6% females were G6PD deficient (Chinevere et al., 2006), signifying that it is X-linked and commonly found in males. In a study carried out by Adenowo and Falusi (2002) to determine accurately the relative frequencies and enzyme activities of polymorphic variants of G6PD in homogenous population in Nigerians, it was stated that 23.9% of males and 4.6% of females were G6PD enzyme deficient, a statement which further supports the understanding that G6PD enzyme deficiency is X-linked and is more likely to be found in males than females (Ademowo and Falusi, 2002).

The distribution of this deficiency tallies with that of malaria, a point that indicates that Zambia must have similar prevalencies as Nigeria due to the endemicity of malaria as suggested by many scholars like Cappellini et al (Cappellini and Fiorelli, 2008). It affects all races but is most common in those of African, Asian, and Mediterranean descent (Gari et al., 2010). This is also confirmed by Dors, who reported that the G6PD deficiency prevalence is increasing in Netherlands due to the immigration of people from Middle East and Africa, especially sub-Saharan Africa (Dors et al., 2008). Howes et al documented that the highest median prevalence, peaking at 32.5% was predicted across sub-Saharan Africa and the Arabian Peninsula (Howes et al., 2012), a factor that indicates high prevalence rates of G6PD deficiency.

In other parts of Africa, especially in the northern parts, the prevalence rate of G6PD deficiency ranges from 0 to 5%. The high rates of G6PD deficiency has a number of diseases that are linked to it. This is as a result of the importance of the enzyme in certain biological systems (Serpa et al., 2010). Using the above statistics for Southern Africa, one would argue that Zambia could have prevalence rates of that magnitude or even higher. To support this statement, other studies have stated that, the prevalence rates of this enzyme deficiency increases as you move towards the sub-Saharan countries, and that it is associated with those areas that are prone to malaria, of which Zambia is one of the areas. A study conducted by Beutler and Duparc in 2007, established

that G6PD deficiency is relatively common in populations exposed to malaria (Beutler and Duparc, 2007).

However, it should be stated that most of the literature has not given the picture of the role that G6PD deficiency plays in essential hypertension development despite the enzyme being among the major antioxidant enzymes that tend to reduce the oxidative stress that many authors have associated with the development of essential hypertension. There are several causes of essential hypertension; these can be, environmental or genetic (Tepel, 2003). This research paper however, looked at G6PD deficiency which is one of the factors that has received minimal coverage when it comes to the development of essential hypertension.

In essential hypertension, oxidative stress may be associated with G6PD deficiency (Rodrigo et al., 2008). The enzyme (G6PD) catalyses the first step in the pentose phosphate pathway. This step leads to the conversion of $NADP^+$ to NADPH + $H^+$, a co-factor that participates in the regeneration of GSH. This reduction of GSSG to GSH takes place in a reaction catalysed by glutathione reductase (Hecker et al., 2013). The reduced GSH then, reduces $H_2O_2$ to $H_2O$, a reaction catalysed by glutathione peroxidase. By this mechanism, the structure of red blood cells is maintained. There is also a balance between the reactive oxygen species (ROS) and antioxidants. Thus, the oxidative stress is in minimal amounts and does not have adverse effects on the red blood cells and the development of essential hypertension (Rodrigo et al., 2008).

The illustration in figure 1.1 shows the role of G6PD enzyme in the reduction of oxidative stress through the inactivation of the free radicals and other reactive oxygen species.

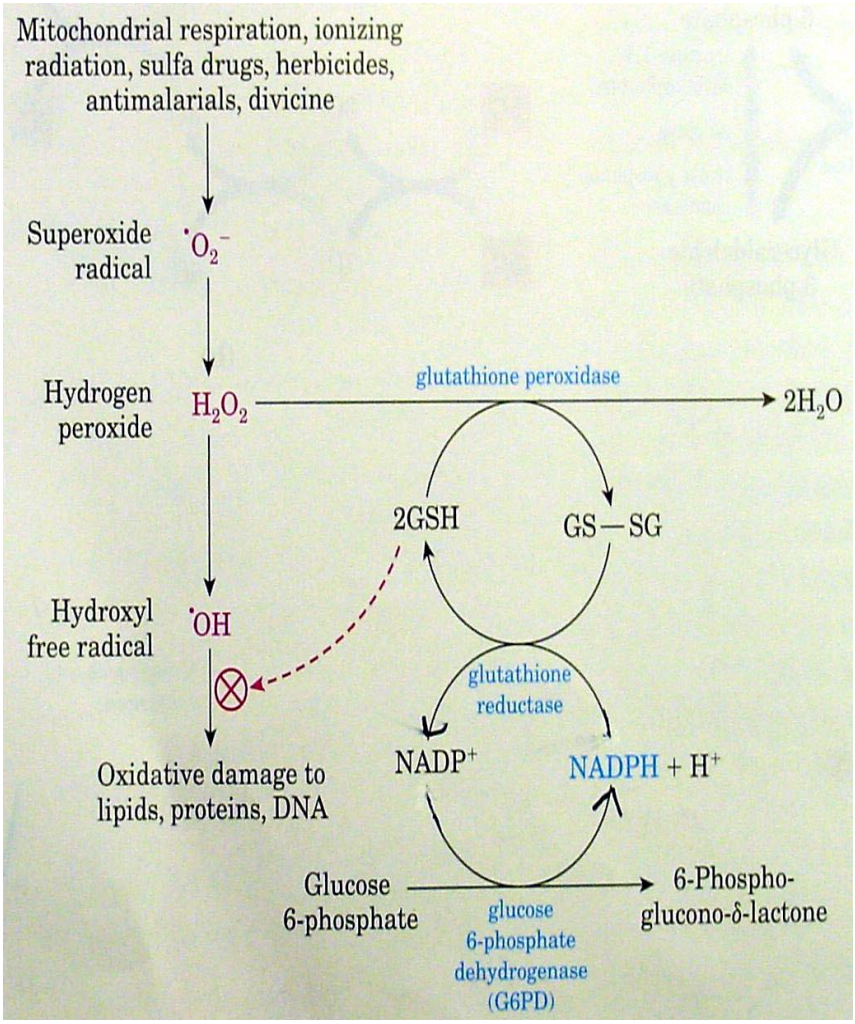



*Figure 1.1:* The effect of G6PD; on the recycling of Glutathione.

Source: Principles of Biochemistry; Leninger.

There are several consequences that are associated with the deficiency of G6PD in the human body system;

1. The effects on the regulation of the activity of the KU protein implied in repairing the DNA after the damage caused by radiation. The G6PD deficiency inactivates KU DNA end binding during stress. KU is an abundant nuclear protein with an essential function in the repair of DNA double-strand breaks (Ayene et al., 2002).

2. Those in the early development of an embryo. When there is a severe deficiency in the extra-embryonic tissues, the placenta development stops and the embryo dies.

3. Those regarding the survival of the fetus during the transition of the fetal haemoglobin to the adult form. Here, the G6PD prevents the oxidative damage due to generation of reactive oxygen species from the adult haemoglobin.

4. The role it plays in phagocytosis. The severe deficiency of this enzyme results in the reduction of the NADPH generation, which results in the decreased reduction of hydrogen peroxide production leading to increased ROS (Martins et al., 1986).

5. In the modulation of vascular endothelial growth factor that regulates the angiogenesis. NADPH is used as a cofactor in the synthesis of nitric oxide (NO) by endothelial nitric oxide synthatase (eNOS) (Merta et al., 2003).

There are various roles of NO; role in inflammation, immune system, nervous system, and a role in blood flow. NO relaxes the smooth muscles in the walls of arterioles, at each systole, the complex endothelial cells that line the blood vessels, will release a puff of NO, which then diffuses in the underlying smooth muscle cells. This process causes these cells to relax, which permits a surge of blood to pass through easily (Gonenc et al., 2013).

A study by Caimi et al, indicated that in human essential hypertension, ROS may increase due to the diminution of the antioxidant enzymes like G6PD. The importance of ROS in vascular function and the development of hypertension have been recently reviewed. It is known that superoxide rapidly inactivates the endothelium derived NO, the most important endogenous vasodilator, thereby, promoting vasoconstriction (Caimi et al., 2010).

Increased vascular stress could be involved in the pathogenesis of hypertension, a major risk factor for cardiovascular disease mortality (Hecker et al., 2013). Oxidative stress occurs when there is an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defence systems so that the latter become overwhelmed (Guindo et al., 2007), a situation that contributes to the development of essential hypertension.

There is another contributory factor that is believed to be associated with essential hypertension. The deficiency in G6PD reduces the availability of NADPH, which diminishes the reduction of GSSG to GSH. This implies that there will be a rise in the ROS due to the reduction in the antioxidant enzymes. This factor tends to later increase the oxidative stress that is believed to

play a role in the development of essential hypertension (Rodrigo et al., 2003). However, there is limited scientific evidence that associates G6PD deficiency with essential hypertension. The only fact that has been known however, is that oxidative stress is one of the cardinal contributors to this essential hypertension. Hence, in red blood cells with sufficient G6PD enzyme there is likely to be less oxidative stress. Dogra reported that the red blood cells of glucose-6-phosphate dehydrogenase (G6PD) deficient person, are unable to scavenge these free radicals that tend to increase the oxidative stress (Dogra et al., 2010).

The build-up of the ROS inactivates NO. The inactivation of NO occurs when superoxide anions and NO react at diffusion-limited rate. This bi-radical reaction can be harmful in at least two ways, first by removing the beneficial effects of NO, and second by producing a highly toxic product, peroxynitrite ($ONOO^-$). Nitric oxide is also affected in the bio-synthesis stage, due to the fact that, the co-factor (NADPH) that acts together with oxygen is decreased. This decrease of NADPH is as a result of the G6PD deficiency. In a study undertaken by Gaskin, oxidative stress and impairment of nitric oxide (NO) are regarded as causative factors of essential hypertension (Gaskin et al., 2001). He went on to say that G6PD generates NADPH, a co-factor in the synthesis of nitric oxide (NO), therefore, when there is G6PD deficiency in granulocytes, there is impairment in the production of nitric oxide (Gaskin et al., 2001). However, Caimi concluded in the study that looked at nitric oxide metabolites and oxidative stress in mild essential hypertension that none of the parameters including nitric oxide was statistically related to the metabolic parameters or to the blood pressure values (Caimi et al., 2010).

In the same study, Gaskin, gave a possible explanation of how G6PD deficiency brings about hyperglycaemia in the polyol pathway. The increased glucose concentration is subjected to non-energy enzymatic production of advanced glycosylation end products (AGE) leading to an increase in superoxide anions, thus quenching the levels of nitric oxide (Gaskin et al., 2001). They also stated that people with X-linked chromosome defects of G6PD deficiency are at risk of getting essential hypertension with persistent ingestion of refined carbohydrates (Gaskin et al., 2001).

Although much of the literature suggests oxidative stress being a major contributory factor to the development of essential hypertension, it has been also established that, the oxidative stress may be as a result of G6PD deficiency, since the reduction of hydrogen peroxide, free radicals and other forms of oxidative stress ultimately and heavily depend on G6PD for the generation of

NADPH (Luzzatto, 1995). Therefore, it can be argued that with the deficiency of G6PD, essential hypertension may be aggravated or made worse.

There are various methods that are used to detect this enzymopathy. The definitive diagnosis of G6PD is based on the estimation of the enzyme activity, by quantitative spectrophotometric analysis of the rate of production of $NADPH + H^+$ from $NADP^+$. For rapid population screening, several semi-quantitative methods have been applied, such as the dye-decolouration test developed by Motulsky in 1961, and fluorescent spot tests, which indicate G6PD deficiency when the blood spot fails to fluoresce under ultraviolet light.

The cytochemical staining assay is another method that is based on the intracellular reduction of the tetra nitro blue tetrazolium (TNBT) by the G6PD via exogenous electron carrier 1-methoxyphenazine methosulfate and TNBT is reduced to dark-coloured water-insoluble formazan, which can be determined by light microscopy (Gurbuz et al., 2005). However, this method cannot quantify the G6PD deficiency, this particular property does not make it suitable for this particular study. The advantage of cytochemical staining is that, it is fast and able to be used on larger studies like surveys of the G6PD deficiency.

In measuring the activity of G6PD, results are affected by episodes of acute haemolysis, presence of high reticulocyte count, in which higher G6PD activity is recorded due to the young erythrocytes and therefore leads to false negative results for G6PD deficiency (Cappellini and Fiorelli, 2008). Difficulties are also encountered in the assessment of neonates, who have a young blood-cell population. However, this was not a problem in this study, as the study was dealing with adults who may only be prone to acute haemolytic conditions and infections. This was however, screened for before participants could be enrolled in the study, and blood was stored at the recommended temperature to prevent haemolysis.

None of the screening tests can diagnose heterozygous females reliably. However, the aim of this study was just to determine whether someone was G6PD deficient or not. The only method that is able to give a definitive diagnosis in females who are heterozygous is the molecular analysis (Cappellini and Fiorelli, 2008). The reason why this molecular analysis method was not selected is due to lack of resources. The molecular analysis can be done after these screening methods have been employed like what Carvalho indicated in his evaluation of G6PD deficiency and neonatal jaundice. A prevalence of 4.6% was reported in new-borns, a higher proportion being observed in those of African descent (Carvalho et al., 2011). A low prevalence in that

study could have been due to the high population of young red blood cells as indicated by Cappellini's study (Cappellini and Fiorelli, 2008).

This study utilised the spectrophotometric method (Pointe scientific Inc. diagnostic procedure, 2001) as it is more reliable than cytochemical and fluorescent spot methods as reported by Ainoon et al., 2003, where it was demonstrated that there was a high false negative rate when using cytochemical and fluorescent spot tests. Ainoon et al also reported that 3.4% of the G6PD deficient neonates were missed by the routine fluorescent spot test and all those that were missed were females (Ainoon et al., 2003).

To gain insights into the link between G6PD and high blood pressure, this study was needed. The study was designed to determine the prevalence rates of G6PD deficiency in essential hypertensive and normotensive adults; establish whether there is a relationship between the G6PD deficiency and essential hypertension, and also determine the levels of nitric oxide and its relationship with essential hypertension.

# Chapter 3

## 3.1 METHODOLOGY

## 3.2 STUDY DESIGN

An analytical cross-sectional study design was used to determine the prevalence of G6PD deficiency.

## 3.3 STUDY SETTING

The study was conducted at the University Teaching Hospital (UTH). This hospital is the largest hospital in the Zambia, and is a tertiary hospital that provides treatment services and medical check-ups for most of the population in Lusaka including referral cases from other provinces.

## 3.4 TARGET POPULATION

The target population were all hypertensive adults aged 35-65 years and normotensives of the same age groups who were coming to the filter clinic to seek medical attention between October, 2013 and January, 2014.

## 3.5 STUDY POPULATION

All adults aged 35-65 years who met the inclusion criteria were recruited in this study.

## 3.6 INCLUSION CRITERIA

1. Adults of both sexes between the ages of 35 and 65
2. Willingness to participate in the study
3. Participants with essential hypertension

## 3.7 EXCLUSION CRITERIA

1. Pregnancy
2. Obesity (BMI >30 $kg/m^2$)
3. Evidence of other chronic diseases like diabetes mellitus
4. Participants with acute haemolysis
5. Patients showing secondary causes of hypertension

### 3.8 SAMPLE SIZE CALCULATION

Based on an expected prevalence of essential hypertension of 50% and 6% G6PD deficiency in normotensives, we enrolled 89 participants from each group in order to have 80% power to detect a 14% difference in G6PD deficiency prevalence (20%) in hypertensive patients, using α=0.05. The sample size was calculated as follows;

$$N = \frac{\left[U\sqrt{\pi_1(1-\pi_1) + \pi_0(1-\pi_0)} + v\sqrt{2\pi(1-\pi)}\right]^2}{(\pi_0 - \pi_1)}$$

Where:

N = size of each group

$\pi_0$, $\pi_1$ Proportions, $\pi$= Average of the proportions

u = 0.84 for 80% power

v = Z statistic = 1.96 if α =0.05

$$N = \frac{\left[0.84\sqrt{0.06(1-0.06) + 0.2(1-0.2)} + 1.96\sqrt{2(0.13)(1-0.13)}\right]^2}{(0.20-0.06)^2}$$

$$= \frac{0.3908 + 0.9322}{0.0196}$$

$$= \frac{1.75}{0.0196}$$

=89.29

=89 participants from each Group.

## 3.9 SAMPLING METHOD

Systematic random sampling method was employed; by selecting every 3rd person was selected for the study. This was done after a thorough medical examination by the medical doctor on duty. The hypertensive participants were recruited from filter clinic at UTH as they came to seek medical attention and normotensive persons were also recruited from the same clinic at the out-patient department. The normotensives were those participants coming to the filter clinic for medical check-ups or minor ailments. The participants were only allowed to take part in the study after showing normal haematological tests and urinalysis.

It should be mentioned here that venous blood amounting to 4mls was collected from the antecubital vein for purposes of determining glucose-6-phosphate dehydrogenase activity (method indicated in the standard operating procedure, page 17), and blood levels of nitrite/nitrates (see procedure on page 22), urea and electrolytes. Urine samples were collected for routine urinalysis.

## 3.10 DATA COLLECTION

The information collected was recorded in the data sheet as a data tool and finally entered in STATA version 12 for analysis. All the study participants went through a thorough medical examination to determine their general health and exclude those with chronic diseases. The physical findings from both groups were recorded in a special examination form (annex 1.3); the information included any antihypertensive drugs being taken by those in the hypertensive group, age, sex, blood pressure and other information that aimed at excluding those with chronic diseases.

## 3.11 VARIABLES

**Table 3.1: Measurement of variables**

| Type of Variable | Definition of the Variables | Scale of Measure |
|---|---|---|
| **Dependent Variables**<br>• Essential hypertension | Essential hypertension- (participants with blood pressure that is ≥140mmHg systolic and ≥90 mmHg diastolic) | Categorical |
| **Independent variables**<br>• G6PD activity<br>• Nitric oxide<br>• Sex<br>• Age | G6PD activity-(measured in U/g Hb at $37^{O}$C, with reference range of 10.01-14.19 U/g Hb) | Categorical |
| | Nitric oxide (NO)- (measured using the levels of Nitrite/Nitrate levels) | Continuous |
| | Sex-(Gender) | Categorical |
| | Age-(Age at last birthday) | Continuous |
| | | |

Table 3.1 shows the variables that were measured in this study. The outcome variable was essential hypertension (categorical variable) and independent variables were G6PD activity (categorical variable), Nitric oxide (continuous variable), age (continuous variable), and sex (categorical variable).

## 3.12 STUDY LIMITATIONS

This study had some limitations worth mentioning. Due to lack of financial limitations, participants in this study could not undergo diagnostic procedures such as; ultrasonography, x-ray, ECG, and endocrinological tests, that were supposed to establish any complications of hypertension.

The physicians however, did a thorough job to rule out various secondary causes of hypertension that could act as confounders in this study. The laboratory results were also used to further confirm the health status of the participants. Since the study was done at the University Teaching

Hospital, only high risk patients sent to the hospital were enrolled, and this may not reflect the true picture of the general population.

### 3.13 STANDARD OPERATING PROCEDURE FOR G6PD ACTIVITY TEST

The G6PD activity was measured using the spectrophotometric method that is outlined in details on the subsequent pages; Glucose-6-phosphate dehydrogenase (G6PD, D-Glucose-6-Phosphate: Oxidoreductase) catalyses the first reaction in the pentose phosphate pathway, oxidising glucose-6-phosphate (G-6-P) to 6-phosphogluconate (6-PG) reducing $NADP^+$ to NADPH + $H^+$. This procedure is a modification of the spectrophotometric methods of Kornberg and Horecker (1948) and of Lohr and Waller, involving the following reaction:

$$\text{G-6-P} + NADP^+ \longrightarrow \text{6-PG} + \text{NADPH} + H^+$$

Nicotinamide adenine dinucleotide phosphate ($NADP^+$) is reduced by G6PD in the presence of G6P. The rate of formation of NADPH is proportional to the activity of G6PD and is measured spectrophotometrically as an increase in the absorbance at 340 nm. Production of the second molar equivalent of NADPH by erythrocyte 6-phosphogluconate dehydrogenase (6-PGDH) is prevented by use of maleimide, an inhibitor of 6-PGDH. This occurs according to the following reaction:

$$\text{6-PG} + NADP^+ \longrightarrow \text{Ribulose-5-Phosphate} + \text{NADPH} + H^+ + CO_2$$

The activity of G6PD is determined in terms of U/g Hb or as $U/10^{12}$ erythrocyte (RBC). In the latter case, the Hb concentration or Red cell count was determined prior to the performance of the G6PD assay.

**REAGENTS**

G6PD R1 Reagent: The reconstituted reagent contained $NADP^+$, 1.5 mM, and maleimide, 12mM, buffer, stabilizer and lysing agent.

G6PD R2 Reagent: Glucose-6-phosphate, 1.05 mM, buffer and magnesium salt. Sodium azide added as preservative.

G6PD controls: Lyophilised controls with G6PD in a stabilised human red cell hemolysate base. Pointe Scientific catalogue number G7583-CTL.

G6PD Lyse reagent: Triton X-100, 0.05% v/v. for use with discrete analyser applications. Pointe Scientific catalogue number G7583-LYS.

**STORAGE AND STABILITY**

1. When unopened, R1 and R2 reagent vials were stored at 2-8$^{O}$C and were stable until the expiration date on the label.
2. Reconstituted R1 reagent is stable for 8 hours at room temperature (18-26$^{O}$C) or 5 days refrigerated (2-8$^{O}$C)

**PRECAUTIONS**

1. It was noted that these Reagents were to be used for in vitro diagnostic only.
2. Normal precautions were exercised in handling the laboratory reagents as follows. Waste was disposed following the laws of the country regarding the waste management.
3. R1 Reagent is harmful: May cause sensitization after inhalation and skin contact. Protective clothing were used to protect the handlers of the reagents.
4. R2 reagent contained sodium azide which reacts with lead and copper plumbing to form highly explosive metal azides. Azide accumulation was therefore avoided.

The preparation of these reagents was done according to the manufacturer's instructions.

**SPECIMEN COLLECTION AND STORAGE**

1. The specimens were collected in accordance with standard procedure.
2. Briefly 4ml of whole blood was collected in EDTA tubes and stored at 6$^{O}$C.
3. Red cell G6PD is stable in whole blood for one week refrigerated at 2-8$^{O}$C, but is unstable in red cell hemolysates. Hence the analysis was done within one week of collection.
4. Freezing blood is not recommended hence the blood samples were not subjected to freezing conditions.

Since the activity of G6PD is reported in terms of grams haemoglobin or the number of red blood cells, the haemoglobin concentration or red blood cell count was determined prior to the G6PD assay. The integrity of erythrocytes collected in acid-citrate-dextrose (ACD) is preserved even after prolonged storage so that obtaining accurate red blood cell counts usually poses no problem. However, red cell counts on specimens collected in heparin become unreliable after 2 days. Thus, for heparinised samples, the results are reported in terms of haemoglobin concentration.

**INTERFERING SUBSTANCES**

1. Copper completely inactivates G6PD at a concentration of 100 µmol/l, and sulphate ions (0.005mol/L) decrease the observed levels of G6PD activity.
2. Reticulocytes have higher G6PD levels than mature red blood cells. Hence the assay was not done after severe haemolytic crises, since G6PD levels may appear falsely elevated. Under these conditions, detection of deficiency may require some family studies.
3. Under normal circumstances, activity contributed by leukocytes, platelets and serum is relatively small. However, in cases of extreme anaemia grossly elevated white counts or, very low levels of red cell G6PD activity, the contribution to the total made under these conditions may be significant.

**TEST PROCEDURE**

The first step was to prepare working R1 reagent by adding the lysate as the diluent instead of $dH_2O$. This reagent was used as follows at $37^OC$:

1. Preparation of the mixture:
   (a) An amount of 1.0 ml of working reagent was added to a well labelled cuvette according to the participant's ID number (which was indicated on the test tube containing the blood sample).
   (b) An addition of 0.01ml of blood was added to R1 and thoroughly mixed to completely suspend erythrocytes. This was let to stand at room temperature ($25.0^OC$) for 10 minutes.
   (c) Then 2.0 mls of R2 reagent was added and mixed gently by inverting several times.

2. The cuvette was then placed in a water bath at $37^{O}$C, to allow for incubation to take place for approximately 5 minutes.
3. Absorbance (A1) of the TEST at 340 nm vs water was then read and recorded using Pharmacia Biotech ultrospec 2000 spectrophotometer. The cuvette was returned to the water bath.
4. Exactly 5 minutes later, the absorbance (A2) was read and recorded.
5. The determination of the G6PD activity was done as indicated in the interpretation of results part.

**CALIBRATION**

The procedure was standardised on the basis of the millimolar absorptivity of NADPH, which is 6.22 at 340 nm. The measurement of the rate of increase in absorbance (ΔA) at 340 nm served to quantitate enzymatic activity.

**INTERPRETATION OF TEST RESULTS**

Before these results were interpreted, the activity of G6PD was calculated as follows:

$$\Delta A\ per\ = \frac{A2 - A1}{5}$$

G6PD activity was expressed as U/g (Hb)

$$\text{G6PD (U/g Hb)} = \Delta A\ per\ min \times \frac{100 \times 3.01}{0.01 \times 6.22 \times Hb(g/dl)} \times TCF$$

$$= \Delta A\ per\ min \times \frac{4839}{Hb(g/dl)} \times TCF$$

Where: 100 = factor to correct activity to 100.0 ml

3.01= Total reaction volume (ml)

0.01= Sample volume (ml)

6.22= Millimolar absorptivity of NADPH at 340 nm

Hb (g/dl) = Haemoglobin Concentration of each specimen

TCF= Temperature correction factor (1 at $37.0^{O}C$)

The reference range for G6PD activity measured at $37.0^{O}C$ was 10.01-14.19 U/g (Hb)

**QUALITY CONTROL**

Reliability of the test results were monitored by use of control materials with known values within each run. Pointe scientific Inc. diagnostic procedure of G6PD activity test came with controls suitable for the above purpose.

G6PD controls: Lyophilised controls with G6PD in a stabilised human red cell hemolysate base. Pointe Scientific catalogue number G7583-CTL.

**MATERIALS**

1. Spectrophotometer capable of measuring absorbance at 340 nm with temperature controlled cuvette compartment was used to measure the absorbance (water bath was used).
2. Pipetting devices for delivery of volumes required for the assay.
3. Cuvettes with optical properties suitable for use at 340 nm.
4. Urine specimen containers: for storage of urine.
5. Urinalysis strips: for screening for any abnormalities of the renal system.
6. Reagents for urea and electrolytes.
7. BP machine: for measuring blood pressure of participants.
8. Thermometer: for temperature readings during physical examinations.
9. Bathroom scale: for taking weights that helped in the BMI calculations.
10. Tape measure was used to measure height of participants to enable BMI calculations.

## 3.14 DETERMINATION OF NITRIC OXIDE

Since NO is not stable, it was mainly measured using the Serum Nitrite/Nitrates concentration, this gave the picture about the status of the Nitric Oxide. The reagents in this procedure were as follows:

1. Reagent A (12.0 mls)
2. Reagent B (500.0 mls)
3. Reagent C (12.0 mls)
4. NaOH (1.0 ml)
5. $ZnSO_4$ (1.0 ml)
6. Standard (1.0 ml)

The first step in this procedure was to deproteinise the serum samples;

1. A volume of 150.0 µL of serum was mixed with 8.0 µL of $ZnSO_4$ in 2.0 mls tubes.
2. The mixture was vortexed and then 8.0 µL of NaOH again was added and centrifuged for 10 minutes at 14 000 rpm.
3. A volume of 100.0 µL of the clear supernatant was transfered to a clean tube.

NOTE: The standards were the same way as the samples to eliminate the need for dilution factor.

The procedure that was selected was a 96 well plate method.

1. Standards: A volume of 500.0 µL of 100.0 µL pre-mix standard was prepared: (A mixture of 50.0 µL of 1.0 µM standard and 450.0 µL of distilled water was prepared.). After which dilution of standards in 2.0 mls centrifuge tubes were done as follows:

**Table 3.2. Procedure for diluting standards in 2.0 mls centrifuge tubes**

| **Tube No** | **Pre-mix Standard + Water** | | **Nitrite (µM)** |
|---|---|---|---|
| 1 | 250.0 µL | + 0.0 µL | 100.0 |
| 2 | 150.0 µL | + 100.0 µL | 60.0 |
| 3 | 75.0 µL | + 175.0 µL | 30.0 |
| 4 | 0.0 µL | + 250.0 µL | 0.0 |

2. i). Reaction: an addition of 100.0 µL of each deproteinised sample was made to separate epperndorf tubes. This was done in duplicates as recommended.

   ii). Immediately prior to starting the reaction, enough working reagent was prepared for all the standards and samples by mixing in the following ratio:

   100.0 µL Reagent A

   4.0 µL Reagent B

   100.0 µL Reagent C

   iii). An addition of 200.0 µL of the working reagent was added to each sample and standard tube and incubated at $60^{O}$C for 10 minutes.

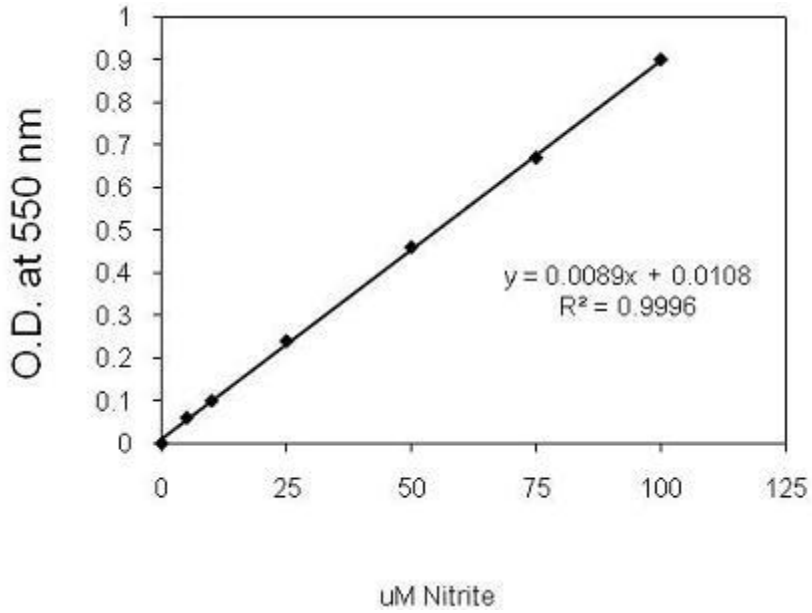
   iv). Measurement: the reaction tubes were centrifuged to pellet any condensation and transferred 250.0 µL of each reaction mixture to separate wells in a 96 well plate.

**CALCULATIONS:**

The OD of the BLANK, which is STD 4 was subtracted from the OD of the standard values i.e., STD 1, 2, and 3. A standard curve was used to get the concentrations of nitric oxide levels (see Fig. 3.1).

Conversion: 1.0 mg/dL Nitric Oxide equals 333.0 µM, 0.001% or 10 ppm.

**Fig 3.1 Standard curve in 96 well plate assay.**



### 3.15 DATA ANALYSIS

This section deals with the methods of data analysis. A total of 178 participants were recruited which accounted for 89 from each group (study and control groups) and information about sex, age, and blood pressure readings was collected using the physical examination form (annex 1.3), after which, participants' blood was analysed to check for G6PD activity, blood levels of urea, creatinine, random blood sugar, and electrolytes. A thorough urinalysis was conducted and results entered on the data sheet for analysis.

Data was analysed using STATA® Version 12 (STATA Corporation, College Station, Texas). The first step in this section dealt with summary statistics for continuous variables for both groups (study and control groups). Mainly, means and standard of deviation were used to come up with the descriptive statistics for continuous variables. The second step involved using frequency tables to establish the prevalence of G6PD deficiency in both groups i.e, study group and the control group. After which three way cross tabulations were done to establish the prevalence distribution of G6PD deficiency among different age groups and gender (male and female) in both groups (study and control group).

A core set of background variables that are believed to influence essential hypertension such as gender, age, G6PD deficiency and nitric oxide levels were defined. Then the significance of these factors was first tested using a bivariate logistic regression to determine the effect of each independent variable on the dependant variable (essential hypertension), then a multivariate logistic regression was performed to establish the effect of the independent variables (G6PD deficiency, gender, nitric oxide levels and age) on the dependent variable (essential hypertension). Then the odds ratios were used to establish the degree of association.

In this study the dependent variable was a dichotomous variable bearing values of '1' if the patient was essential hypertensive and '0' if they were normotensive. Logistic regression was used due to this binary nature of the outcome variable. Specifically, the logistic regression model considers the relationship between a binary dependent variable and a set of independent variables. The logistic model for k independent variables ($X_1, X_2, X_3, \ldots, X_k$) is given as:

$$\text{Logit } p(x) = \alpha + \sum \beta_i X_i$$

Where;

Exp ($\beta_i$) = Odds ratio for a person having characteristic i versus not having characteristic i

β=Regression coefficient.

α=Constant

The results of the logistic regression were interpreted using odds ratio (OR). An odds ratio (OR) is a measure of association between an exposure and an outcome. The OR represents the odds that an outcome will occur given a particular exposure, compared to the odds of the outcome occurring in the absence of that exposure. Odds ratios are most commonly used in case-control studies (Szumilas, 2010). The interpretation of the odds ratios would be as follows;

OR=1 Exposure does not affect odds of outcome (No real relationship between the outcome and predictor variable).

OR>1 Exposure associated with higher odds of outcome (odds of outcome occurring increases).
OR<1 Exposure associated with lower odds of outcome (Any increase in the predictor leads to a drop in the odds of the outcome occurring.)

## 3.16 ETHICAL CONSIDERATIONS

Prior to the commencement of this study, ERES CONVERGE reviewed and approved the protocol. Permission was also granted by University Teaching Hospital. The information gathered from participants was strictly confidential. Assurance was given that information would be disseminated to relevant authorities and with no direct link to the participants, since anonymity was maintained. The results would also be used for improvement of the health status of the patients that may be affected by this condition.

The study participants were recruited based on their willingness to participate in the study, they were also informed through a consent form (annex 1.1, 1.2) about the pain they were going to experience during blood collection. Accuracy was ensured by training the nurses that were handling the collection of blood from participants.

# Chapter 4

## 4.1 RESULTS

This section presents results that firstly indicate whether there is a difference in prevalence rates of G6PD deficiency between the study group and control participants. Secondly, the findings show the distribution of G6PD deficiency prevalence among gender and age groups. Lastly, the association of G6PD deficiency, nitric oxide and essential hypertension is determined using the multivariate logistic regression.

## 4.2 CHARACTERISTICS OF PATIENTS WITH ESSENTIAL HYPERTENSION AND CONTROL PARTICIPANTS.

Summary statistics for the study and control group are presented in Table 4.1. The study group consisted of 89 hypertensive patients with a mean age and standard deviation of 52.6 and 10.4 respectively greater than those in the control group (mean age: 43.8± 9.0 years) and the difference was significant (*P<0.0000*). Other variables whose means came out to be significant at 5% include serum nitric oxide (mean 70 ± 39.9 µM for the study group and 96.5 ± 48.6 µM), glucose (mean 5.2 ± 0.8 mmol/L (study group), 5.0 ± 0.9 mmol/L (control group), creatinine (mean 0.7 ± 0.1 mg/dl (study group), 0.7 ± 0.2 mg/dl) and sodium levels (mean 136.6 ± 1.5 mmol (study group), 137.1 ± 2.3 mmol (control group) all with p-values less 0.05. The data also indicated a higher prevalence of G6PD deficiency (16%) in the study group compared to the control (10%), though the difference was not statistically significant (*P = 0.13*).

**Table 4.1. Descriptive statistics for Essential hypertensive patients and control participants.**

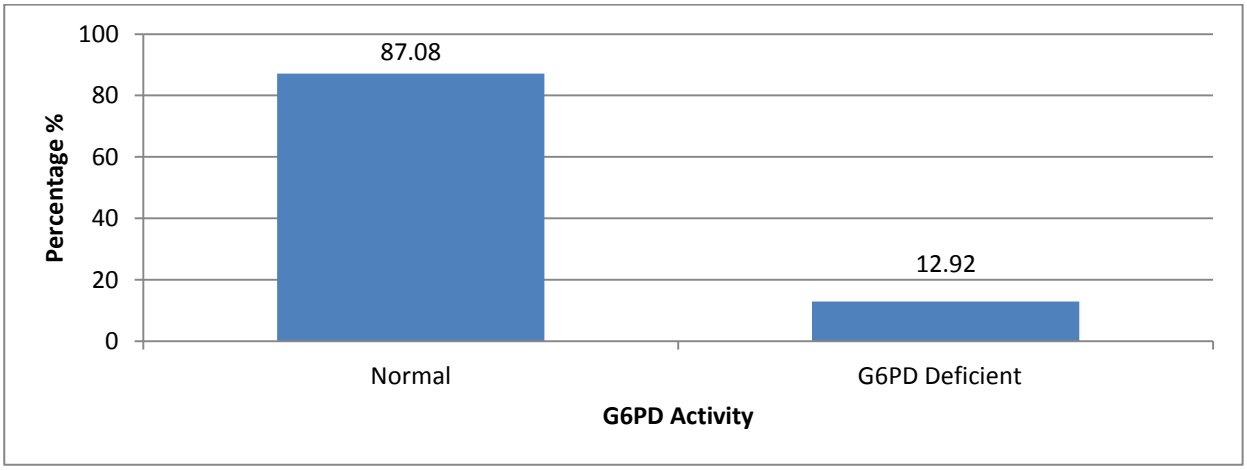
| | Essential hypertensive Patients (study group) ( n=89) | | Normotensive (control group) (n=89) | | P-values (cases vs. controls) |
|---|---|---|---|---|---|
| | Mean | Standard deviation | Mean | Standard deviation | |
| Age (Years) | 52.1 | 10.4 | 43.8 | 9.0 | <0.0000 |
| Nitric oxide (µM) | 70.0 | 39.9 | 96.5 | 48.6 | <0.0001 |
| pH | 6.6 | 0.3 | 6.6 | 0.3 | 0.25 |
| Specific Gravity | 1.0 | 0.0 | 1.0 | 0.0 | 0.07 |
| Glucose (Blood )(mmol/L) | 5.2 | 0.8 | 5.0 | 0.9 | 0.03 |
| Urea (mmol/L) | 3.8 | 1.1 | 3.6 | 0.9 | 0.08 |
| Creatinine (mg/dl) | 0.7 | 0.1 | 0.7 | 0.2 | 0.05 |
| Sodium (mmol) | 136.6 | 1.5 | 137.1 | 2.3 | 0.03 |
| Potassium (mmols) | 3.7 | 0.2 | 3.7 | 0.2 | 0.40 |
| Chloride (mmols) | 100.0 | 2.6 | 100.2 | 3.0 | 0.39 |
| G6PD deficiency, n (%) | 14 (15.7) | | 9 (10.11) | | 0.13 |

Source: Authors' own analysis, 2014

## 4.3 GLUCOSE-6-PHOSPHATE DEHYDROGENASE ENZYME DEFICIENCY PREVALENCE IN ESSENTIAL HYPERTENSIVE AND NORMOTENSIVE ADULTS.

The study recruited 89 participants from each group; that is, study and control groups making a total of 178 participants. Out of these, 23 participants were G6PD deficient; representing a prevalence rate in the whole group of 13%; and 155 participants had normal activity of the enzyme G6PD; representing 87%. Figure 4.1 shows the distribution of the G6PD deficiency.

**Figure 4.1. Percent distribution of G6PD activity in the study Sample (n=178)**




**Source: Authors' own analysis, 2014**

Table 4.2 shows the distribution of G6PD deficiency by age category. Out of all those that were G6PD deficient in the study group, 50% belonged to the age group of 35-45, 42.86% belonged to 46-55 age group, and 7.14% belonged to 56-65 age group, while all the participants in the control group who were G6PD deficient belonged to 35-45 age group.

**Table 4.2. G6PD deficiency by age category**

| | Study Group | | Control Group | |
|---|---|---|---|---|
| | G6PD deficiency | Normal G6PD activity | G6PD deficiency | Normal G6PD activity |
| Age category | ----------%---------- | | | |
| 35-45 | 50 | 30.67 | 100 | 62.5 |
| 46-55 | 42.86 | 16 | | 20 |
| 56-65 | 7.14 | 53.33 | | 17.5 |
| TOTAL | 100 | 100 | 100 | 100 |

**Table 4.3. G6PD prevalence by gender**

| | Study Group | | Control Group | |
|---|---|---|---|---|
| | G6PD deficiency | No G6PD deficiency | G6PD deficiency | No G6PD deficiency |
| | ----------%---------- | | | |
| Gender | ---------. | | | |
| Female | 7.14 | 54.67 | 33.33 | 45 |
| Male | 92.86 | 45.33 | 66.67 | 55 |
| TOTAL | 100 | 100 | 100 | 100 |

Source: Study data, 2014

Table 4.3 presents the prevalence of G6PD deficiency by gender of the participants. As highlighted, G6PD deficiency is more prevalent in males than in females as can be seen both in the study and control groups although the prevalence rate in the former (93%) is greater than in the latter (67%).

## 4.4 THE RELATIONSHIP BETWEEN G6PD DEFICIENCY, NITRIC OXIDE AND ESSENTIAL HYPERTENSION

A bivariate logistic regression was performed to determine the effect of each independent variable on the dependent variable (essential hypertension), after which a multivariate logistic regression was done to control for potential confounders.

**Table 4.4. Bivariate logistic regression with Essential Hypertension as the dependent variable with unadjusted odds ratios (Number of cases =89; Number of controls=89)**

| Variables | | P-Value | Unadjusted OR (95% CI) |
|---|---|---|---|
| G6PD | Not deficient | 0.27 | 1.00 |
| | Deficient | | 1.66 (0.68-4.07) |
| Age category(in years) | 35-45 | 0.05 | 1.00 |
| | 46-55 | | 2.21 (0.99-4.96) |
| | 56-65 | | 5.76 (2.72-12.21) |
| Gender | Female | 0.65 | 1.00 |
| | Male | | 0.87 (0.48-1.58) |
| NO levels (µM) | - | 0.00 | 0.98 (0.98-0.99) |

Key: OR-Odds Ratio, NO-Nitric Oxide, G6PD-Glucose-6-Phosphate Dehydrogenase.

Table 4.4 presents the results for the unadjusted odds ratio for the bivariate logistic regression. The results indicate that only age and the levels of nitric oxide have a significant effect on hypertension at 5% significance level. More specifically, participants in 46-55 age group were 2.2 (95% CI 0.99-4.96) times more likely to develop essential hypertension than those in 35-45 age group; and those in 56-65 age group were 5.7 (95% CI 2.72-12.21) times more likely to develop essential hypertension than participants in 35-45 age group. The findings also indicate a significant association between nitric oxide levels and essential hypertension with the odds ratio showing a decreased risk for essential hypertension [OR]=0.99, 95% [CI]=0.48-1.58, *p=0.00.*

**Table 4.5. Logistic regression for predictors of development of essential hypertension among 35-65 year olds screened at UTH.**

| Variables | | Found with essential hypertension after screening. | | Unadjusted OR (95% CI) | Adjusted OR (95% CI) |
|---|---|---|---|---|---|
| | | Yes n (%) | No n (%) | | |
| G6PD | Not deficient | 75(84.3) | 80(89.9) | 1.00 | 1.00 |
| | Deficient | 14(15.7) | 9(10.1) | 1.66 (0.68-4.07) | 2.92 (1.07-7.95) |
| Age category(in years) | 35-45 | 30(33.7) | 59(66) | 1.00 | 1.00 |
| | 46-55 | 18(20.2) | 16(18) | 2.21 (0.99-4.96) | 2.41 (1.05-5.54) |
| | 56-65 | 41(46.1) | 14(15.7) | 5.76(2.72-12.21) | 7.31 (3.22-16.61) |
| Gender | Female | 42(47.2) | 39(43.8) | 1.00 | 1.00 |
| | Male | 47(52.8) | 50(56.2) | 0.87 (0.48-1.58) | 0.79 (0.39-1.60) |
| NO levels (µM) | - | - | - | 0.98 (0.98-0.99) | 0.98 (0.97-0.99) |

Key: OR-Odds Ratio, NO-Nitric Oxide, CI- Confidence Interval and G6PD-Glucose-6-Phosphate Dehydrogenase.

Source: Author's analysis, 2014.

Table 4.5 reveals that age, and G6PD deficiency all are positive and significantly associated with increased risk of hypertension, while nitric oxide is associated with reduced risk of essential hypertension. The participants in 46-55 age group were 2.4 (95% CI 1.05-5.54) times more likely to develop essential hypertension than those in 35-45 age group; and participants in 56-65 age group were 7.3 (95% CI 3.22-16.61) times more likely to develop essential hypertension than those in 35-45 age group. The results further indicate that participants with G6PD deficiency were 2.92 (95% CI 1.07-7.95) times more likely to develop hypertension than those without the G6PD deficiency, while an increase in nitric oxide levels was more likely to lead to decreased risk of having essential hypertension ([OR]=0.99, [CI]=0.98-0.99.

# Chapter 5

## 5.1 DISCUSSION

The study explored the prevalence of G6PD deficiency in essential hypertensive and normotensive adults aged between 35 to 65 years and its association to essential hypertension. It also investigated the relationship between nitric oxide and essential hypertension. This section provides answers to the above specific objectives of this study.

The study found that G6PD deficiency prevalence was at 16% in the study group, which translated to 14 participants out of 89 recruited participants in this group. While in the control group, the prevalence was at 10% translating to 9 participants out of 89. Although there seems to be a difference of 6%, the p-value indicated no statistical significance (*p=0.13*). Comparing the control group's prevalence rates of 10% to other studies, there are similarities in the rates with the findings of Luzzatto's study (Luzzatto, 1995) which established 4 to 26% prevalence of G6PD deficiency in health Nigerian individuals. In a similar study carried out by Egesie in a healthy male Nigerian population, the findings showed a 20% G6PD deficiency prevalence. The WHO (2008) also suggested a 15-26% in individuals without any condition but in malaria endemic zones. From this, we can establish that the findings are in line with what other authors have established. There is however paucity of data regarding the G6PD deficiency prevalence in individuals with essential hypertension. Although this is the case, it is known for a fact that people with X-linked chromosome defects of G6PD deficiency are at risk of developing essential hypertension (Gaskin et al., 2001). For this reason, it was hypothesised that the prevalence of G6PD deficiency was higher in essential hypertensive participants than in normotensive people, though the difference in G6PD deficiency prevalence was statistically not significant according to the findings of the present study. The G6PD deficiency prevalence in the study group could not be compared to other studies because many studies regarding G6PD deficiency have been carried out in healthy individuals as highlighted above.

In a study carried out to establish the prevalence rates of G6PD deficiency in 490 new-born jaundiced babies, Carvalho et al., (2011) reported a 4.6% of G6PD deficiency, a prevalence that is within the range 4-26% that Luzatto (1995) found in a study of a Nigerian health population. This further cements the fact that prevalence rates of G6PD deficiency are expected to be in the same range in normotensive and essential hypertensive people. But what may differ is probably the susceptibility to certain conditions when one is G6PD deficient. The research findings therefore suggest that there is no statistical difference in the G6PD deficiency prevalence

between essential hypertensive and normotensive adults. The study further proceeded to look at the association between G6PD deficiency and essential hypertension.

A multiple logistic regression was performed to determine the effect of G6PD deficiency on essential hypertension as the dependent variable. Using the odds ratio (OR), the results indicated a positive association between the G6PD deficiency and essential hypertension. It was found that a G6PD deficient individual was 2.9 times more likely to have essential hypertension than a non-G6PD deficient individual. This is in agreement with what Luzatto (1995) stated about oxidative stress. The author stated that, oxidative stress, which is a major contributor to essential hypertension, arise from G6PD deficiency since the reduction of hydrogen peroxide, removal of free radicals and other forms of oxidative stress to a large extent depend on G6PD enzyme for the generation of NADPH. In a study conducted by Gaskin et al (2001), the author also stated that people with X-linked chromosome defects of G6PD deficiency are at risk of getting essential hypertension. Rodrigo et al (2003), in their study, pointed out that the reduction of anti-oxidant enzymes such as G6PD enzyme leads to the generation of oxidative stress that is likely to increase the risk of having essential hypertension. It can therefore be concluded from the findings of this study that, G6PD deficiency has a possible role in the development of essential hypertension.

The third specific objective was to establish the relationship between nitric oxide levels and essential hypertension. In order to do this, a multivariate logistic regression was performed where the odds ratio was established to be 0.99 (OR<1). This means that, nitric oxide is associated with lower odds of essential hypertension. This implies that an increase in the nitric oxide levels reduces the likelihood of one developing essential hypertension ([OR]=0.99). In a study done by Rodrigo (2003), in titled implications of oxidative stress and homocysteine in the pathophysiology of essential hypertension, the author commented to say that hypercysteinemia limits the bioavailability of nitric oxide, thereby increasing chances of having essential hypertension through the increase of oxidative stress. From this we can conclude that NO is important in the maintenance of normal blood pressure and its increase will reduce the likelihood of one developing essential hypertension. The findings therefore, corroborate with what other studies have suggested about the relationship between nitric oxide and essential hypertension. It can therefore be concluded from the study that there is an association between nitric oxide levels and essential hypertension, where an increase in the NO levels will lead to reduced risk of having essential hypertension.

In our study, age was also found to have a positive association with essential hypertension, with the participants in 45-55 age group being 2.4 times more likely to have essential hypertension than those in 35-45 age group, on the other hand, participants in 56-65 age group are 7.3 times more likely to have essential hypertension than those in 35-45 age group. This means that the increase in age is associated with higher odds of essential hypertension. This is supported by a study conducted by Goma et al (2001), on the prevalence of hypertension and its correlates in Lusaka Urban District of Zambia, where he found that age and sex (gender) were associated with hypertension. In this study, however, it was found that sex had no association with essential hypertension [OR]=0.79, [CI]=0.39-1.60 (*P=0.6*).

This study had some challenges worth mentioning. The spectrophotometric method could not determine the prevalence of hemizygote state in females who present with normal G6PD activity but are reservoirs in the community for the abnormal gene. For future studies, it would be worthwhile to consider doing a molecular analysis after spectrophotometry.

## CONCLUSION

This study establishes the G6PD deficiency prevalence in the essential hypertensive adults (study group) to be 15.7% (16%), and 10.1% (10%) in normotensive adults. However, there is no statistical difference in the prevalence of G6PD deficiency between the two groups ($p=0.13$). Therefore it can be concluded that there is no difference in G6PD deficiency prevalence between essential hypertensive and normotensive adults seen at UTH during the study. However, the present study showed a possible role of G6PD deficiency in the pathophysiology of essential hypertension. To answer the third specific objective, the findings demonstrated an association between nitric oxide levels and essential hypertension. It can therefore be concluded that; an increase in nitric oxide is associated with decreased risk of developing essential hypertension, whereas G6PD deficiency is associated with increased risk of having essential hypertension.

It remains to be elucidated whether essential hypertension is caused by a defect in endothelial nitric oxide synthesis or by an impaired vascular response to nitric oxide. If indeed a defect in synthesis of NO was the cause of essential hypertension perhaps clinical trials could be recommended where administration of supplements, which upon metabolism release NO, would be given in essential hypertension. Additional studies will be required to determine the nitric oxide levels in hypertensives with normal and low glucose-6-phosphate dehydrogenase activity; and in normotensives with normal and low glucose-6-phosphate dehydrogenase activity.

## RECOMMENDATIONS

1. This study has shown relatively high prevalence rates of G6PD deficiency in both groups, therefore, nation-wide G6PD deficiency screening programmes should be carried out to ascertain the prevalence rates for the general population, as this study only carried out the analysis on participants coming to University Teaching Hospital. This will help in the management of essential hypertension bearing in mind the possible role that G6PD deficiency plays in the development of essential hypertension, a finding that was established in this study.

   In addition to the above recommendation, molecular tools can be incorporated to determine the deficient participants reliably, and severity of the G6PD deficiency.
2. The findings of this study indicated that, with increase in NO, there will be reduced risk of being essential hypertensive; therefore NO supplementation which plays a role in the maintenance of blood pressure can be encouraged thereby, helping in the management of essential hypertension.
3. The study indicated that those found deficient of G6PD enzyme are at higher risk of having essential hypertension due to the compromised antioxidant status that protects one from effects of oxidative stress; therefore, individuals who are essential hypertensive and G6PD deficient, could be encouraged to take antioxidants such as fruits, and vegetables as a means of reducing the oxidative stress, which adversely leads to essential hypertension.

## REFERENCES


ABOLGHASEMI, H., MEHRANI, H. & AMID, A. 2004. An update on the prevalence of glucose-6-phosphate dehydrogenase deficiency and neonatal jaundice in Tehran neonates. *Clin Biochem,* 37**,** 241-4.

ADEMOWO, O. G. & FALUSI, A. G. 2002. Molecular epidemiology and activity of erythrocyte G6PD variants in a homogeneous Nigerian population. *East Afr Med J,* 79**,** 42-4.

AINOON, O., ALAWIYAH, A., YU, Y. H., CHEONG, S. K., HAMIDAH, N. H., BOO, N. Y. & ZALEHA, M. 2003. Semiquantitative screening test for G6PD deficiency detects severe deficiency but misses a substantial proportion of partially-deficient females. *Southeast Asian J Trop Med Public Health,* 34**,** 405-14.

BEUTLER, E. & DUPARC, S. 2007. Glucose-6-phosphate dehydrogenase deficiency and antimalarial drug development. *Am J Trop Med Hyg,* 77**,** 779-89.

CAIMI, G., MULE, G., HOPPS, E., CAROLLO, C. & LO PRESTI, R. 2010. Nitric oxide metabolites and oxidative stress in mild essential hypertension. *Clin Hemorheol Microcirc,* 46**,** 321-5.

CAPPELLINI, M. D. & FIORELLI, G. 2008. Glucose-6-phosphate dehydrogenase deficiency. *Lancet,* 371**,** 64-74.

CARVALHO, C. G., CASTRO, S. M., SANTIN, A. P., ZALESKI, C., CARVALHO, F. G. & GIUGLIANI, R. 2011. Glucose-6-phosphate-dehydrogenase deficiency and its correlation with other risk factors in jaundiced newborns in Southern Brazil. *Asian Pac J Trop Biomed,* 1**,** 110-3.

CASTRO, S., WEBER, R., DADALT, V., TAVARES, V. & GIUGLIANI, R. 2006. Prevalence of G6PD deficiency in newborns in the south of Brazil. *J Med Screen,* 13**,** 85-6.

CHINEVERE, T. D., MURRAY, C. K., GRANT, E., JR., JOHNSON, G. A., DUELM, F. & HOSPENTHAL, D. R. 2006. Prevalence of glucose-6-phosphate dehydrogenase deficiency in U.S. Army personnel. *Mil Med,* 171**,** 905-7.

DOGRA, N., PURI, G. D. & RANA, S. S. 2010. Glucose-6-phosphate dehydrogenase deficiency and cardiac surgery. *Perfusion,* 25**,** 417-21.

DORS, N., RODRIGUES PEREIRA, R., VAN ZWIETEN, R., FIJNVANDRAAT, K. & PETERS, M. 2008. [Glucose-6-phosphate dehydrogenase deficiency: clinical presentation and eliciting factors]. *Ned Tijdschr Geneeskd,* 152**,** 1029-33.

EFFERTH, T., SCHWARZL, S. M., SMITH, J. & OSIEKA, R. 2006. Role of glucose-6-phosphate dehydrogenase for oxidative stress and apoptosis. *Cell Death Differ,* 13**,** 527-8; author reply 529-30.

EGESIE, O. J., JOSEPH, D. E., ISIGUZORO, I. & EGESIE, U. G. 2008. Glucose-6-phosphate dehydrogenase (G6PD) activity and deficiency in a population of Nigerian males resident in Jos. *Niger J Physiol Sci,* 23**,** 9-11.

GARI, M. A., CHAUDHARY, A. G., AL-QAHTANI, M. H., ABUZENADAH, A. M., WASEEM, A., BANNI, H., AL-SAYES, F. M., AL-HARBI, A. & LARY, S. 2010. Frequency of Mediterranean mutation among a group of Saudi G6PD patients in Western region-Jeddah. *Int J Lab Hematol,* 32**,** 17-21.

GASKIN, R. S., ESTWICK, D. & PEDDI, R. 2001. G6PD deficiency: its role in the high prevalence of hypertension and diabetes mellitus. *Ethn Dis,* 11**,** 749-54.

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. *Int Arch Med,* 4**,** 34.

GUINDO, A., FAIRHURST, R. M., DOUMBO, O. K., WELLEMS, T. E. & DIALLO, D. A. 2007. X-linked G6PD deficiency protects hemizygous males but not heterozygous females against severe malaria. *PLoS Med,* 4**,** e66.

GUPTE, R. S., VIJAY, V., MARKS, B., LEVINE, R. J., SABBAH, H. N., WOLIN, M. S., RECCHIA, F. A. & GUPTE, S. A. 2007. Upregulation of glucose-6-phosphate dehydrogenase and NAD(P)H oxidase activity increases oxidative stress in failing human heart. *J Card Fail,* 13**,** 497-506.

GURBUZ, N., AKSU, T. A. & VAN NOORDEN, C. J. 2005. Biochemical and cytochemical evaluation of heterozygote individuals with glucose-6-phosphate dehydrogenase deficiency. *Acta Histochem,* 107**,** 261-7.

HECKER, P. A., LEOPOLD, J. A., GUPTE, S. A., RECCHIA, F. A. & STANLEY, W. C. 2013. Impact of glucose-6-phosphate dehydrogenase deficiency on the pathophysiology of cardiovascular disease. *Am J Physiol Heart Circ Physiol,* 304**,** H491-500.

HO, H. Y., CHENG, M. L. & CHIU, D. T. 2007. Glucose-6-phosphate dehydrogenase--from oxidative stress to cellular functions and degenerative diseases. *Redox Rep,* 12**,** 109-18.

HO, H. Y., CHENG, M. L., LU, F. J., CHOU, Y. H., STERN, A., LIANG, C. M. & CHIU, D. T. 2000. Enhanced oxidative stress and accelerated cellular senescence in glucose-6-phosphate dehydrogenase (G6PD)-deficient human fibroblasts. *Free Radic Biol Med,* 29**,** 156-69.

HOWES, R. E., PIEL, F. B., PATIL, A. P., NYANGIRI, O. A., GETHING, P. W., DEWI, M., HOGG, M. M., BATTLE, K. E., PADILLA, C. D., BAIRD, J. K. & HAY, S. I. 2012. G6PD deficiency prevalence and estimates of affected populations in malaria endemic countries: a geostatistical model-based map. *PLoS Med,* 9**,** e1001339.

LUZZATTO, L. 1995. About hemoglobins, G6PD and parasites in red cells. *Experientia,* 51**,** 206-8.

LUZZATTO, L. 2012. G6PD deficiency and malaria selection. *Heredity (Edinb),* 108**,** 456.

PEREZ-CRESPO, M., RAMIREZ, M. A., FERNANDEZ-GONZALEZ, R., RIZOS, D., LONERGAN, P., PINTADO, B. & GUTIERREZ-ADAN, A. 2005. Differential sensitivity of male and female mouse embryos to oxidative induced heat-stress is mediated by glucose-6-phosphate dehydrogenase gene expression. *Mol Reprod Dev,* 72**,** 502-10.

RODRIGO, R., PASSALACQUA, W., ARAYA, J., ORELLANA, M. & RIVERA, G. 2003. Implications of oxidative stress and homocysteine in the pathophysiology of essential hypertension. *J Cardiovasc Pharmacol,* 42**,** 453-61.

RODRIGO, R., PRAT, H., PASSALACQUA, W., ARAYA, J. & BACHLER, J. P. 2008. Decrease in oxidative stress through supplementation of vitamins C and E is associated with a reduction in blood pressure in patients with essential hypertension. *Clin Sci (Lond),* 114**,** 625-34.

SERPA, J. A., VILLARREAL-WILLIAMS, E. & GIORDANO, T. P. 2010. Prevalence of G6PD deficiency in a large cohort of HIV-infected patients. *J Infect,* 61**,** 399-402.

TEPEL, M. 2003. Oxidative stress: does it play a role in the genesis of essential hypertension and hypertension of uraemia? *Nephrol Dial Transplant,* 18**,** 1439-42.

# 1.0 ANNEX

## 1.1 INFORMATION SHEET

**Title of Study; A comparative study of the prevalence of glucose-6-phosphate dehydrogenase deficiency in essential hypertensive and normotensive adults aged between 35 and 65 years at the University teaching hospital, Lusaka, Zambia.**

Dear Participant,

This is to inform you about the study and request you to take part in this study that is being carried out by Dr Mwenya Kwangu C., who is a student at the University of Zambia, School of Medicine, Department of Physiological Sciences. The study will focus on high blood pressure which has been on the increase in Zambia. Several causes have been associated to the rise in the cases. However, 95% of the cases are as a result of high blood pressure with unknown cause. Therefore, efforts should be made in reducing the problem minus the use of conventional drugs which have side effects.

To start with, a physical examination will be carried out by qualified and registered medical doctors working at the University Teaching Hospital (UTH) (i.e., clinic 5 and filter clinics) to determine your health status, this physical examination will require 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.

Then 4mls of blood; and urine will be collected from you. The collection of these samples will only be done once (i.e., at the start of the study). The following tests will be performed on all blood samples; test to determine nitric oxide, Glucose-6-phosphate dehydrogenase enzyme activity, electrolytes, blood sugar levels, creatinine, and urea. The urine samples will be used for routine urinalysis using the strips.

The following are the known risks of this procedure of collecting blood; hematoma-when collecting blood samples, blood may leak out of a vein (blood vessel) and under the skin and form what is called hematomas; there can also be swelling, tenderness, and inflammation at the site of blood collection, at times there is vasovagal responses such as; dizziness, sweating, coldness of skin, numbness and tingling of feet, nausea, vomiting, possible visual disturbance, syncope(fainting) and injury fall from fainting, allergy may also present itself where the collection site starts itching or burning (rashes or hives may form near the site), there are also rare risks like; close up (thrombosis) of the blood vessel (vein) due to trauma, and infection which results in close up of the blood vessel and inflammation (thrombophlebitis). Special

precautions that will be put in place to reduce these risks are to use sterile equipment and alert the medical doctors on call in case of adverse effects; prolonged swelling and wounds as a result at the collection site will treated using antibiotics and drugs to reduce pain and swelling, however you should not worry because you will be attended to by well qualified doctors and nurses attached to the University Teaching Hospital.

There will be no direct or monetary gain to you by participating in this research. The blood examination will be used to gather information which will contribute to management of high blood pressure with unknown cause. Your participation in this study is purely voluntary and therefore, you are eligible to withdraw if you are not interested and your action will not affect your acquisition of health services.

Please seek clarification where you do not understand. All the information you will provide will be strictly confidential. The research information will be disseminated to the relevant authorities and with no direct link to you since anonymity shall be maintained.

## 1.2 INFORMED CONSENT FORM

The purpose of the study has adequately been explained to me and I understand the aim, benefits, risks and confidentiality of the study. I further understand that; if I agree to take part in this study, I can withdraw at any time without having to give an explanation and that taking part in this study is purely voluntary.

I…….agree to provide blood and urine samples for use by…….

**I have been informed of the following:**

1. The quantity of blood to be taken…… (Specify the amount).
2. The quantity of urine to be donated…….. (Specify amount).
3. The number of times of blood and urine donations…….**.....** (Specify the number of times).
4. The use that will be made of the samples…….**.....** ……...

I agree to the samples being stored for possible further uses in future YES/NO

Participant's signature……Date……

Signature of person taking blood……...Date……

## A. PERSONS TO CONTACT FOR PROBLEMS

1. Mwenya Kwangu C. (Dr), University of Zambia, School of Medicine, Department of Physiological Sciences, P.O. Box 50110, Lusaka, Zambia. Mobile Phone; 0977 234327.

2. The Chairperson, ERES Converge, 33 Joseph Mwilwa Road, Rhodes Park, Lusaka, Tel: +260 955 155 633, +260 955 155 634, +260 966 765 503, Email: eresconverge@yahoo.com.

### 1.1 Icishibisho pali ili sambililo (Information sheet in BEMBA)

**Umutwe we sambililo; isalambililo lya kwishiba ukucepa kwa glucose-6-phosphate dehydrogenase muba lwele ba BP nabo aba shakwata ubu bulwele, abali nemyaka 'yakufyalwa ukwambila pa 35 ukufika ku 65. Ili sambililo, likacitikila pa cipatala cikalamba icabela mu musumba wa lusaka muno mwine mucalo cesu ica Zambia.**

Kuli imwe ba kacele wandi,

Ndemishibishako pali ili sambililo no kumilomba ukutila mwingasendamo ulubali, muli ici ,cisambililo icile citwa na 'musambi uweshina lya Mwenya Kwangu (Dr) uli kumasambililo ya pamulu pe sukulu likalamba iyitwa University of Zambia, pe sukulu ya kusambilila ubumi bwa bantu. Ici cisambililo cikalolekesha pa bulwele bwa BP ubo ubulekulilakofye inshita ne nshita. Kwaliba ifintu ifingi ifingalenga ubu bulwele ukufula mu bantu. Ukulanda fye icishinka, iikalamba 'mpendwa ya ubu bulwele bwa BP, bu lengwa ne fintu ifi shaishibikwa. Palici, amaka yafwile ukubikwako pakuti ubu ubwafya bwa cepanako mu calo cesu ica Zambia, nga cingacitwa, ubu bulwele bwa fumishiwapo ukwabula ukubomfya ishi miti shamufipata ishakwatako ubusanso ngashabomfeshewa panshita itali.

Icakwambilapo, ukucecetwa kwa ku mubili kwalacitwa na ba shing'anga abo abalembeshiwa na 'kamfulumende yesu kaili aba bomba pacipatala cikalamba (UTH) mutupatala tunono utwitwa clinic 5 na filter. 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 nangula abaume, mufwile ukulanda amalwele ayo mwalwalapo, namalwele yapalupwa, kabili mufwile mwalondolola ifyo mumfwa limo-limo, nangula ngakwaliba ifimicusha mumubili wenu, ba shing'anga balayesha kabili no kumiceceta konse-konse, ukwambila kumutima, amolu, iminofu nemimonekele ya muli wenu.

Panuma yaku cecetwa, umulopa ukulingana nama mls ayali yane (4mls) eyalasendwa ukufuma kuli baimwe, ukulundapofye, mwalafwaikwa ukupelako nemisu mukakunkubiti akanono eko balamipela. Ukukupela kwaifi fintu kwalafwaikwa ukucitwafye umukumo mpo fye. Umulopa ana 'misu mwala tupela fikabomba ngefi: kumulopa; tukesha ukwishiba ubwingi bwa nitric oxide, Glucose-6-phosphate,

electrolytes, sugar, urea, na creatinine. Imisu nasho shikabomba mukusanga ifintu ifingalufyana ubumi bwamuntu.

Ifi, efintu fimo ifingamicitila ilyo mule pela umulopa; umulopa ukwikalila panshi ya nkanda, ukufimba pancende yakufumishapo umulopa, ukumfwa ulunshingwa, ukupiba, ukumfwa ukutalala, icifungalashi, umuselu, ukuluka, ukufulunganishiwa mu kulolesha, ukufwapo icitu, no kuicena ilyo umuntu awa pa mulandu wa kufwapo icitu, ukupumbuka utupumba pa ncende pantu inyeleti yacingila mukufumya umulopa, ukucenwa ku mishipa iisenda umulopa mumibili, ico icingalenga iyi mishipa ukwisalika no kulesha ukupita kwa mulopa, lelo ici, tacicitika sana-sana iyo.
Mukwesha ukucefya amasanso aya; ifyela ifya samfiwa bwino efikaba ifyakubomfeshiwa. Kucilishapofye, abo bonse abakasangwa abacushiwa pamulandu wa wakupela umulopa, ba kaba aba undapwa na ba shing' anga abakebwa ilyo icicisambililo tacilapwa. Umuti wa kulesha ubukali na 'tushishi ukwingilila pacilonda ca nyeleti ukalapelwa kuli abo bonse abakafimba ukutwala mu bulwele. Mwisakamana pantu mulimumaboko yaba kanye kanye, ba bumi, abo abalamitangata ubusaka sana. Aba ba shing'' nga na ba nurse bantu aba bomba pacipatala cikalamba ica UTH.
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 'ci, takwacilinganishiwe nakalya awe.
Twapapata mukwai, apo tamumfwikishe, ipusheni, batila ati, kabusha, takolelwe bowa, no waluba nawo tabamuseka iyo. 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.

**1.2 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.

Ine…….nasumina ukupela umulopa na ‘misu ukubomfeshewa naba ……...

**Nabanjishibisha palifi:**

1. Ubwingi bwamulopa ndepeela…… (lembeni ubwingi).
2. Ubwingi bwamisu ndepeela……(lembeni ubwingi ).
3. Imiku ya kupeela imisu na ‘mulopa……..... (lembeni imiku).
4. Incito iyo umulopa na ‘misu iikabomba……. …….

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

Icishibilo ca ba kapela……ubushiku……..

Icishibilo ca ba kasenda wa mulopa……...ubushiku……..

## A. ABANTU BAKUMONA NGA MWASHINGWANA NAMAFYA

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**1.1 Uthenga wofotokoza za kufufuza kukuchitidwa (Information sheet in CHINYANJA)**

**Mutu wa Zofufuzidwa:**

**Kusiyanitsa uchepa kwake kwa sugar yochedwa glucose -6-phosphate mu gulu la antu a zaka zakubadwa makhumi atatu ndi mbabu zisanu kufika mpaka zaka zakubadwa makhumi asanu ndi chimodzi ndi mbambu zisanu; odwala matenda othamangitsa mutima komanso aja omwe a mitima zo puma bwino –muchipatala chachikulu UTH (University Teaching Hospital).**

Kwa Otengako mbali mu kufufuza uku,

Ndiku dziwitsani za kufufuza kudzachitidwa ndi a Sing'anga –Dokota (Dr) Kwangu Kwenya C. amdne ali pa maphunzilo apamwamba pa skulu yaikulu (University of Zambia), kupunzila za mankwala ndi kuchilitsa, kumbali ya mayendedwe a zinthu muthupi. Komanso ndi kukupempani kuti mutengeko mbali mu kufufuzaku. Kufufuza uku kudzagogomeza pa za matenda a BP omwe akhala ali kuchuluka mu dziko la Zabiya (Zambia). Kuchuluka kwa matenda a BP mudziko agwilizanidwa ndi zifukwa zingapo. Pantawe zochulukilapo, pafupi-fupi nthawi zones (95%) matenda a BP amawapeza, koma akatwiki samapeza chowayambisa matendawa. Nchifukwa chache ndichaphindo kuyetsetse kupeza njila ina yochepetsela matenda a BP kapena Othamangitsa mutima. Njila inayo iyenela kukhala yopanda zotuluka zapadela potsatila kumwa makwala.

Choyambilila, a kastwili, a sin'ganga (Dotolo/dokotala) olembesedwa ku boma omwe a sebenza nchito ku UTH (University Teaching Hospital) clinic 5 ndi ma clinic osefer adza pima kuti adziwe zatanzi lanu panthawiyo comanso nthawi zakumbuyo. Muyenele ku a dziwisa; zaka zakubadwa, uima kwanu (kapena ndu amuna olo akazi), azafutsanso kuti aziwe za matenda amene banja lanu kapena achibale ana anadwalapo.Azaona zizindikilo za matenda pa thupi panu ndipo azapina kasebenzedwe ka mutima, muchfuba; miyendo, manja nthanze ndiponso zina ndi zina zokhuza thanzi lanu.

Akatha kupima ndikufunsa zones zachulidwa mu ndime pamwambapa; azattenga magazi okwanila kupima ma mls anai (4). Mikozo nayonso izafunika kutengedwa. Zonsezizizachitidwa kamozi chabe (pa masiku oyambilila a kufufuza uku).

Zomwe azapima mu magazi ndi mikodzo yonse idzatengedwa zili motele:

Adzapima nitric oxide, Glucose-6-phosphate dehydrogenase enzyme activity, electrolytes, sugar mu magazi, 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. Ane anthu amanve utenth, mwina unyaula pa malo olasidwa ndonga kapena nsingano. Angono mwa anthu padziko amakhala ndi mwazi omwe umauma musanga. Ntawi zina magazi amauma mopitilila. Magazi yaka uma mu muzipe yamayenda yenda kulondola kapumidwe ka muttima mina kufikila mizzipe kutssekeka chifukwa cha magazi ouma ndi kuvimba kwa mizipe. Ofufuza adziwa za izi ndipo ayesa kuika mumalo zina ndi zina zoyetsetsa kuti zododometsazi zitsachitike. Akumbutsa akatswili apa chipatala chachikulu (UTH) omwe azitenga magazi kuti adziwitse mwamsanga a dotolo kapena a tsin'ganga akaona zizindikilo zo sonyeza chilichonce mwa zododomesazi. Akawadziwitsa, akatwili ndi ma sisita kapena a nasi apa chpatala chachikulu azaona ndi kusamalila zoutulukapo izi; adzapatsa mankwala oleketsa kuvimba ni yena ochotsa tuzilombo twa matenda muthupi kulingana ndi zomwe zizafunike ngati wna aliyense mwa odzipeleka apezeka ndi vuto chifukwa chodzipeleka.

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 kulandila kwa thandizo yamankwala mu chipatala kapena zina zanthanzi lanu siziza pastsidwa mochipekela chifukwa mwakana kutngako mbali. Koma zo phindulapo ndi zakuti, kudzipeleka kwanu, magazi anu opimidwa ndi zina zo peze pakufufuza zidzathandiza kuti matenda o thamanga mutima ("BP") omwe alusa pantawi ino achilitsidwe mposadziwa chifukwa achilitsidwe.

Uthenga, ndi mayankho omwe mudzapatsa pa kufufuza uku, kudzasunidwa mu chinsinsi. Zopeza pa kufufuzaku zizatumidzidwa kumaofesi akulu muchisinsi chamaina komanso zonse zina zowadziwitsa za otengako mbali. Ngati zinna zolembedwa mu ndime izi sizinanveke, chonde funsani.

**1.2 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.

Ine,…….ndivomela kupeleka magazi ndi mikozo…….

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

1. Kuchuluka kwake kapena muyeso wa magazi azatengedwa kukapima…… (lembani muyeso).

2. Muyeso wa mikozo womwe muzaeleka…….. (lembani muyeso).

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

4. A fotocoza zomwe azachita ndi magazi komanso mikodzo omwe udzatengedwa…….....

……...

Ndavomekze kuti angasunge magazi ndi mikozo yotengedwayo kuti mutsogolo, INDE Ai

Chizindikilo cha odzipeleka (wotengako mbali)…….

Tsiku la a Chaka……/……/20……

Chizindikilo cha otenga magazi blood…….……...

Tsiku la a Chaka……/……/20……

A. **Keyala ya owe mungauze ngati mwapeza vuto yokhuzana ndi kuzipeleka kapena kutengakumbali pa kufufuza uku:**

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## 1.3 PHYSICAL EXAMINATION FORM

I.D Number: ……..

Age: ……..

Sex: ……

**PAST MEDICAL HISTORY**

Hypertension……

Diabetes……

Gout…….

Asthma……..

Endocrinological disorders……

Drug history……..

**FAMILY HISTORY**

.....

.....

.....

.....

.....

.....

.....

.....

| **COMMON SYMPTOMS** | **YES** | **NO** |
|---|---|---|
| Headaches | …… | ……. |
| Chest pains | …… | …… |
| Swelling of feet | …... | ……. |
| Difficulties in passing urine | …… | ……. |

If Yes (difficulties in passing urine)…….

……

……

| | | |
|---|---|---|
| Inappetance | …… | …… |
| Abdominal pains | …… | …… |
| Visual Problems | …… | …… |

## GENERAL EXAMINATION

Blood pressure.....

Pulse……..

……...

Pallor…….

……...

Jaundice…….

……..

Pedal Oedema…….

……...

Any other abnormalities……

……

**CARDIOVASCULAR AND RESPIRATORY SYSTEM EXAMINATION**

| | **NORMAL** | **ABNORMAL** |
|---|---|---|
| Heart Sounds | …….. | ……. |

If abnormal,
explain……
……
……
…….

| | | |
|---|---|---|
| Auscultation (Chest) | …… | …… |

If abnormal,
explain……
……
……
……

Inspection, percussion, and palpation
results……

……
……

X-ray
results……
……..

| **GASTROINTESTINAL EXAMINATION** | **YES** | **NO** |
|---|---|---|
| Any organomegally | …… | ……. |

If yes,
explain……..
……..
……..
……...

**CENTRAL NERVOUS SYSTEM EXAMINATION**

| | **NORMAL** | **ABNORMAL** |
|---|---|---|
| Reflexes | …….. | …… |

If abnormal,
explain……
……
……
…….

| | | |
|---|---|---|
| Power in extremities | …… | ……. |

If abnormal,
explain……
……
……
……

**MUSCULOSKELETAL SYSTEM EXAMINATION** **NORMAL** **ABNORMAL**

……
……
……
……
……
……...

If abnormal,
explain……
……
……
……
……

**LABORATORY EXAMINATION**

Urinalysis……
……

Urea……
……

Creatinine……
……

Random Blood
Sugar……
……

Haemoglobin
levels……
……

Other red blood cell
indices……

Thyroid functional
tests……

**STUDY LABORATORY EXAMINATION RESULTS**

| | |
|---|---|
| Glucose-6-phosphate dehydrogenase activity | |
| Serum levels (Nitrite/Nitrates) | |

**PUBLISHED ARTICLES**