CHAPTER ONE: Introduction

1.1 Background

Hypertension, a condition in which the blood pressure in the arteries is chronically elevated, is a common and complex human disease that causes significant morbidity and mortality worldwide. (A.D.A.M. Medical Encyclopaedia. Hypertension).

The prevalence of hypertension in sub-Saharan Africa ranges from 6% to 48% (Delas et al, 2011). Despite recent advances in the understanding and treatment of hypertension, its prevalence continues to rise (Dickson et al, 2006).In a study among adults in urban Lusaka, Zambia, the prevalence of hypertension was found to be 34.8% (38.0% of males and 33.3% of females) (Goma et al, 2011). These prevalence estimates were higher than those reported from South Africa of 25.5% among females and 21.6% for males, and from Uganda of 22.0%and from Eritrea of 16% overall. Compared to the prevalence of hypertension reported in Zimbabwe (Matenga, 2000), these estimates were higher for males (38.0% for Lusaka vs. 26% for Zimbabwe) and lower for females (33.3% for Lusaka vs. 41% for Zimbabwe) (see Goma et al, 2011).

Hypertension, like other Non-Communicable Diseases (NCDs), is associated with identifiable behavioural and biological risk factors. The major risk factors include race, obesity, diabetes, age, sex, alcoholism, sedentary lifestyle, diet (including salt intake), and family history of hypertension. Some of these risk factors for hypertension are modifiable through lifestyle interventions or at least their effects ameliorated by lifestyle modifications and medical management (Goma *et al,* 2011).

Studies of ambulatory blood pressure measurements in twins suggest that essential hypertension has a strong genetic component (Hottenga et al, 2005). Some researchers have suggested that a family history of hypertension is associated with salt sensitivity of blood pressure, implying that this phenomenon may be genetically mediated. Recently, substantial progress has been made in elucidating the molecular mechanisms that

cause several rare forms of hypertension, including hypertension arising from mutations in the 11[beta] - hydroxylase gene, Liddle's syndrome, and glucocorticoid-suppressible aldosteronism. New results have been obtained on the possible role of the angiotensinogen gene, and other candidate genes, such as the angiotensin II receptor Type I gene and the Sa gene, in human hypertension. Investigations of experimental models of hereditary hypertension have also been important in unravelling the genetic complexity of the disease (Soubrier *et al,* 2006).

Haptoglobin genes have been cited as risk factors in the development of hypertension (Hossein, 2004). Haptoglobin is an acute phase glycoprotein that circulates in the blood. The gene has three phenotypes of Hp 1-1, Hp 2-1 and Hp 2-2 (Carter et al, 2007). The association of haptoglobin phenotypes with different clinical conditions has become of great interest to researchers. Haptoglobin phenotype types 1 (Hp 1-1) and 2 (Hp2-2) have been linked to susceptibility to various diseases including diabetes, heart disease and infection (Vlierberghe et al, 2004). Some studies have shown that Haptoglobin (Hp) gene is a major risk factor for diabetes vascular disease (Levy et al, 2003). (Levy, 2003). Haptoglobin polymorphism has been suggested as a candidate genetic marker in essential hypertension (Delanghe et al, 1994). Recent studies in humans have shown that there is an association between haptoglobin (Hp) genes and hypertension (Hossein et al, 2004)(Hossein, 2004); this association however varies between populations (Quaye et al, 2006). However, none of these studies have been done in Africa.

This research seeks to investigate the association between presence of haptoglobin phenotypes and hypertension in patients attending outpatient medical clinic at the University Teaching Hospital, Lusaka.

1.2 Problem Statement

The impact of ethnic and racial differences on hypertension underscores the need for the identification of the genetic factors that contribute to differences in susceptibility to and the pathology of the disease. In the Zambian and most of the African populations, such studies have not yet been done.

1.3 Justification of Study

This study will add to information on the understanding of the association of the haptoglobin (Hp) phenotype and hypertension in Zambia. It will also add to the pool of knowledge on the genetic factors that can be implicated as risk factors in the development and poor prognosis of hypertension.

1.4 General objective

The aim of this study is to investigate the association between haptoglobin serum levels and haptoglobin phenotypes, and hypertension in hypertensive clients and normotensive clients at the University Teaching Hospital (UTH) in Lusaka.

1.5 Specific objectives

1. To establish prevalence of the candidate haptoglobin phenotypes in hypertensive and normotensive subjects.

2. Determine the association between the prevalence of the haptoglobin phenotypes and risk factors for hypertension.

CHAPTER TWO: Literature Review

Haptoglobin structure and types

Haptoglobin is synthesized by hepatocytes (Smithies and Walker, 1995; Bowman, 1993) and there is evidence that suggests that haptoglobin may also originate from the organs of the reproductive system including human uterus (O'Bryan et al, 1997; Olson et al, 1997; Sharpe-Timms et al, 2002). It is an α_2-glycoprotein acute phase reactant that binds to free haemoglobin and forms a stoichiometrically stable complex (Hosein et al, 2004).

The haptoglobin protein has a tetrameric structure consisting of 2α and 2β chains encoded by a single gene on chromosome 16q22.3. These chains are generated by posttranslational cleavage from a single polypeptide (Yang et al, 1983; Ranyuei et al, 1983). The β chains are identical in all individuals, while the α chains are polymorphic (Smithies et al, 1962; Langlois et al, 1996) and found only in humans (Black and Dixon, 1968; Teye et al, 2004; Langlois et al, 1996). Although haptoglobin is found in serum of all mammals, this polymorphism is only found in humans (Bowman et al, 1993). The protein polymorphism is due to two codominant alleles Hp1 and Hp2, which result in three common genotypes Hp1-1, Hp2-1 and Hp2-2. These genotypes give rise to structurally and functionally distinct phenotypes: Hp1-1, Hp2-1 and Hp2-2. Hp1-1 has α1 chains; Hp2-2 has α2 chains whereas Hp2-1 contains both chains (Fig 1) (Koda et al, 2000; Schultze, 1996, Teye et al, 2002; Melamed-Frank, 2001).

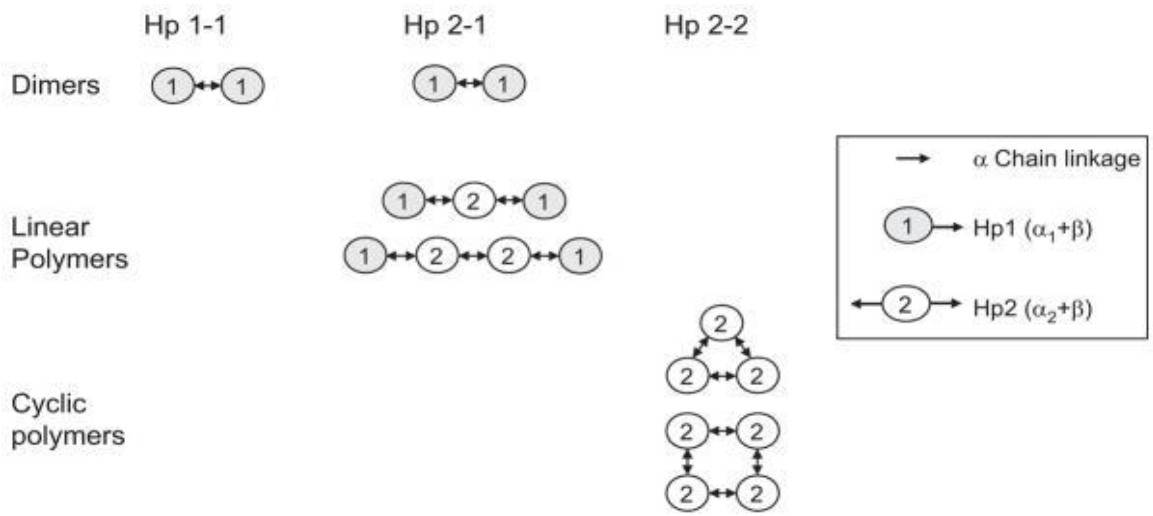


Figure 1. A schematic illustration of the shape of Hp polymers in humans with the Hp 1-1, Hp 2-1 and Hp 2-2 genotypes (Koda et al, 2000).

The α_2 chain contains two free cysteine residues compared to one in the α_1 chain, leading to polymerization in Hp2-1 and Hp2-2, while Hp1-1 is a small monomer (Fig 2) (Wuyts et al, 2002).

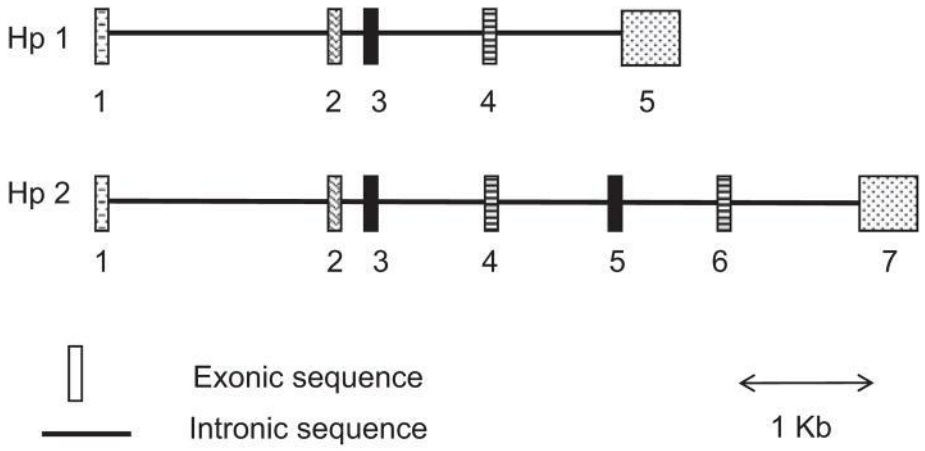


Figure 2. Schematic diagram of the genomic structure of the Hp alleles 1 and 2. (Wuyts et al, 2002).

The fourth phenotype, Hp0, represents hypohaptoglobinemia or anhaptoglobinemia (Giblett et al, 1969). In Hp0 phenotype, the expression of haptoglobin is either absent (anhaptoglobinemia) or very low (less than 15 to 20 mg/100ml) to be detected by gel electrophoresis (hypohaptoglobinemia) (Giblett et al, 1969; Delanghe et al, 1998). Although Hp0 could be caused by pathological states such as liver dysfunction and

haemolytic disorders, there is evidence that this phenotype also has a genetic origin (Koda et al, 1998; Teye et al. 2003, Teye et al, 2004).

Haptoglobin prevalence

Langlois *et al* documented that the gene frequencies of Hp show marked geographical differences, with the lowest Hp1 allele frequency in Southeast Asia and the greatest frequency in Africa and South America (Carter et al, 2007). Carter *et al* (2007) have shown that Africa as a whole has an Hp1 allele frequency of 56% with Hp2 being 44%. For example Ghana had a haptoglobin frequency of Hp1 52% and Hp2 48%. Kenya also had Hp1 at 57% and Hp2 at 43% (Carter et al, 2007).

Haptoglobin and hypertension

The polymorphism of the haptoglobin gene with its various phenotypic expressions makes it have different biological efficiency in its function as a protein in the body. This observation provides a basis for the use of haptoglobin phenotypes as a predictor of susceptibility to cardiovascular disorders, like hypertension, and the patient's prognosis (van Vlierberge et al, 2004).

The best-known function of haptoglobin is haemoglobin binding. After erythrocyte destruction, free haemoglobin is not filtered through the glomeruli because it is bound to haptoglobin. This reduces the risk of renal damage and the loss of haemoglobin and iron. The Hb-Hp complex is transported to the liver, where it is broken down in the parenchymal cells by lysosomes. The binding property of haptoglobin also prevents accumulation of free radicals generated by iron Fe^{2+} in the presence of H_20_2 (van Vlierberge et al, 2004). The complex of Hb-Hp also holds Hb dimer in a ferrous oxidation state that scavenges and inactivates nitric oxide (NO) through deoxygenation reaction thereby inhibiting endothelial-dependent relaxation. This is a contributor to the causation of endothelial dysfunction, which is a risk factor for hypertension (van Vlierberge et al, 2004).

Nitric oxide is a free radical that plays a principal role in basal blood flow regulation and vascular homeostasis. As an NO scavenger, the Haptoglobin–Haemoglobin (Hb-Hp) complex has a role in regulating NO bioavailability and vascular homeostasis (Wang *et al*., 2004). The differences in the structure of Hp1-1, Hp 2-1 and Hp2-2 appear to have clinical significance in hypertension due to the functional differences in protecting against Hb-driven oxidative stress and NO consumption. Hp1-1 is a dimer, and it has a smaller size than the linear Hp2-1 and the cyclic Hp2-2. This enables the polymer Hp1-1 to bind to and clear more molecules of the Hb via monocyte/macrophage scavenger receptors CD163, thus conferring protection against oxidative stress and better consumption of the NO than the other two phenotypes (Asleh R et al, 2003). Therefore, it has been demonstrated that the differences in size of each haptoglobin phenotype may affect both access to tissues and the rate of clearance of the haptoglobin–haemoglobin complex and the subsequent regulation of the bioavailability of the NO (Langlois *et al*., 1997b; Na et al., 2006a). For example it has been demonstrated that the Hb-Hp1-1 complexes are cleared more rapidly than the Hb-Hp2-2 complexes. On the other hand, Hp2-2 phenotype is less efficient in clearing haemoglobin circulating in the plasma than Hp1-1 and Hp2-1 (Langlois et al, 1996). These differences in uptake rates of the Hb-Hp complexes, having a profound effect on NO bioavailabity, bring about alterations in normal blood flow as a result. This may cause turbulence, which contributes to arterial pressure and cardiac thrombosis (Asleh et al, 2003)(Asleh R, 2003).

Hermann M *et al* in their study on Nitric oxide and hypertension observed that impaired NO bioactivity may be the major link between hypertension and further development of cardiovascular disorders (Hermann et al, 2006). Indeed clinical studies have shown that patients with hypertension have a blunted arterial vasodilation response to infusion of endothelial-dependent vasodilators and that inhibition of NO raises blood pressure. Arterial stiffness, a major mechanism of systolic hypertension, is suggested to occur as a result of impaired NO bioactivity (Hermann M, 2006). They further observed that Mice with a disrupted endothelial NO synthase gene had elevated blood pressure levels

compared to control animals. This suggests a genetic link between impaired NO bioactivity and hypertension.

In a study by Surya *et al* (1987) on Haptoglobin patterns in essential hypertension and associated conditions of increased risk for Hp 2-2, they observed that patients with Hp 2-2 phenotype showed a significantly increased risk for essential hypertension and hypertension associated with ischaemic heart disease. There was a significant decrease in the mean levels of serum haptoglobin in hypertension as compared to controls, suggesting the possibility for intravascular haemolysis due to vascular damage leading to further complications of cardiovascular disorders. The association between haptoglobin phenotypes and peripheral vessel disorders has also been observed and studied. The Hp 2-2 phenotype is overrepresented in peripheral occlusive disorders. Haptoglobin, by limiting the availability of haeme compounds to catalyse the oxidation of arachidonic acid by prostaglandin synthetase, inhibits prostaglandin synthesis and so has an anti-inflammatory action (Kamg et al, 1983). The presence of Hp 2-2 predisposes the peripheral vessels to damage.

Haptoglobin phenotypes may also provide a pathogenic link between obesity and hypertension and its other co-morbidities. Serum Hp constitutes a novel marker of adiposity in humans, and the adipose tissue likely contributes to determine its levels (Chiellini et al, 2003). Here, it is speculated that Hp could constitute an important link between obesity and its comorbidities, such as hypertension, by mediating some of the inflammatory effects associated with the obesity status. Furthermore, obesity is associated with insulin resistance and increased rate of cardiovascular events (Uchegbu *et al*. 2002). Of note is the knowledge that Hp has been related to the development of arterial hypertension (Montani *et al.* 2002) and to the incidence of myocardial infarction and stroke (Engstrom, *et al.* 2002). Thus, it is speculated that Hp, which is also overproduced in obesity, might constitute a novel link between obesity and hypertension and cardiovascular disorders.

Plasma haptoglobin levels change during life. Haptoglobin levels in healthy infants are lower than in healthy adults. In healthy adults, the haptoglobin concentration in plasma is between 0.38 and 2.08 g/L. People with Hp 1-1 have the highest plasma concentrations, those with Hp 2-2 the lowest plasma concentrations, and those with Hp 2-1 have concentrations in the middle. In other words, haptoglobin levels may be affected by the Hp phenotype, with circulating concentrations in the following order Hp1-1 > Hp2-1 > Hp2-2 (Langlois et al, 1996; Imrie at al., 2006). According to Imrie H *et al.* 2006, the levels of circulating serum haptoglobin were as follows; Hp 1-1 (0.31mg/mL), Hp 2-1 (0.28mg/mL) and, Hp 2-2 (0.23mg/mL). The model also predicts a difference in median Hp level between the different Hp genotypes with higher levels in 1-1 (0.115 mg/mL) compared with 2-1 (0.057 mg/mL) and 2-2 (0.058 mg/mL). Therefore the levels of serum haptoglobin can be used to determine which phenotype is most predominant in the individual.

No study on the association of haptoglobin phenotypes and hypertension has been done in Zambia yet, let alone the frequency and distribution studies of the haptoglobin phenotypes in the country. The stated prevalence of hypertension of the country (34.8%) is one of the highest in the region (Goma et al, 2011). This then necessitates the finding of potential risk factors, both the genetic and environmental ones, and their possible interactions in seeking to better understand the condition in its pathology and treatment. There is very little literature on the genetic and molecular basis of hypertension in Zambia. If the information is there, it is probably unpublished and inaccessible to the public. The genetic composition and environmental condition differ in our population group from that of other groups who have done the studies on haptoglobin phenotypes associations with hypertension. This underscores the need to localize many of these studies to the Zambian context.

CHAPTER THREE: Methodology

3.1 Study Design

This was a descriptive cross-sectional study involving haptoglobin quantification and phenotyping of hypertensive and normotensive subjects. The normotensive subjects acted as a reference population in the study.

3.2 Study Site/ Sampling of Participants

The study was conducted at the University Teaching Hospital (UTH) in Lusaka, Zambia. Consecutive hypertensive subjects that attended clinic 5 at UTH were enrolled with written consent from each subject, as participation in this study was voluntary.

The study population comprised of 50 Zambian men and women with already clinically diagnosed and known hypertension. Permission was granted to have a look at the subjects' clinical files to verify their hypertension status. Patients who had both diabetes and hypertension were excluded from the study. This is because diabetes poses as a confounder to the investigation of the association. Other healthy subjects were recruited from within the hospital community to act as controls in the study. These had no clinical record of hypertension or diabetes. A total of 100 subjects were recruited for the study.

Inclusion criteria

- Hypertensive patients
- Non-hypertensive controls
- Above 18 years old
- Non diabetic

Exclusion criteria

- Less than 18 years
- Subjects who were both diabetic and hypertensive

3.3 Sample Size Determination

The prevalence of hypertension is about 37% in Lusaka. The sample size of the hypertension group was calculated as follows: From a population of 202 per month three times a month who attend the Medical Clinic at the University Teaching Hospital in Lusaka gives a sample of 606. Using an expected frequency of 37% and a precision of 5% that brings worst acceptable result to 42% at 95% confidence level and 80% power, a sample of 225 hypertension participants would be recruited. The controls: 225 would also be recruited to maintain the 1:1 ratio. Hence the sample size was calculated to be 450. However, for proof of consent and due to limitation of resources, a sample size 50 for each group will be used, hence 100.

3.4 Data Collection

1. Anthropometric measurements:

a. Height

A well-calibrated meter measuring tape was used to measure the height of the participant. Height was measured without the participant wearing foot or headgear. Before the reading was taken, the participant was requested to have feet together, heels against the back board, knees straight, and look straight ahead. Height was recorded in centimetres.

b. Weight

Weight was measured using the Heine Portable Professional Adult Scale 737 (Secagmbh & Co. kg Humburg, German). Participants were asked to take off their footwear and to stand still, face forward, and place arms on the sides of the body. Weight was recorded in kilograms.

Blood pressure Measurement

The nurses attending to these subjects as a routine check-up of the vitals of the patient before seeing the doctor took the blood pressure for the subjects at the medical clinic.

The auscultatory method was used utilising a mercury sphygmomanometer and krotkoff sounds heard at the antecubital artery. Phase V of the krotkoff sounds was used for documentation of diastolic blood pressure. A subject was classified as hypertensive if he or she had repeatedly elevated systolic blood pressure of above 140 mmHg and diastolic blood pressure of above 90 mmHg or was on medication.

Haptoglobin Quantification and phenotyping

The Hp assay was performed on an ABX Pentra 400 analyser (Horiba Medical) using a calibration curve with a top Hp standard of 2.0 mg/mL such that samples with Hp values greater than 2.0 mg/ml were automatically diluted.The ABX Pentra haptoglobin diagnostic reagent for quantitative *in-vitro* determination of haptoglobin in serum and plasma by turbidity was used. The materials required for this analysis were the Haptoglobin reagent, ABX Pentra Protein Calibrator, ABX Pentra Protein Control Low/High, ABX Pentra Accelerator I CP, ABX Pentra Sample diluent CP. The analyser was calibrated and all the controls were run according to standard operating procedure.

The analysis was done according to the Standard Operating Procedures (SOP) for the reagent and test to ensure good performance of the system analyser in the analysis of the samples. The results for the haptoglobin quantity for each study participants were printed out and analysed accordingly.

Haptoglobin phenotyping was done using the mean levels of the phenotypes as follows; Hp 1-1 (1.26 ± 0.43 g/L), Hp 2-1 (1.08 ± 0.50 g/L) and Hp 2-2 (0.84 ± 0.42 g/L) (Langlois et al, 1996). (Langlois M, 1996)The sera concentrations were used to indicate the type of the Hp present in the study participants and thus were classified as Hp 1-1, 2-1 or 2-2.

3.4 Data Entry

Data entry was done using SPSS Statistics version 17.0. Data was doubly entered and validated. The data entry template was checked for consistency and range checks embedded in it. The data was then exported to Statview and Epi data for analysis.

3.5 Data Analysis

The data analysis included the descriptive statistics of the study population of ages, sex, BMI, serum concentrations and blood pressure. The quantitative data were expressed as percentages, and shown as mean ±SD. Student's (t) test was used for comparison of the serum levels between the two groups having quantitative normally distributed data, and One way analysis of Variance (ANOVA) test was used for comparison between three or more groups having quantitative normally distributed data. The Pearson chi-square test was used to compare the qualitative variables to test the statistical significance for the association of hypertension with the haptoglobin polymorphisms. The allele frequencies were calculated for a two-allele system and differences in haptoglobin genotype and subtype frequencies between groups were compared using Pearson chi-square test. P-value was considered statistically significant when it is less than 0.05.Unadjusted odds ratios (OR) and their 95% Confidence interval (CI) were done and presented. In the analysis, body mass Index (BMI) was categorized as <18.5 kg/m^2 (lean), 18.5-24.9 kg/m^2 (normal), 25.0-29.9 kg/m^2 (over weight), and 30+ kg/m^2 (obese).

3.6 Ethical considerations

The University of Zambia (UNZA) Biomedical Research Ethics Committee (REC) reviewed the survey protocol and granted ethical approval. Informed consent was obtained from each of the study participants. Venous blood was drawn from consented participants and it was made clear and understandable that this would cause some discomfort and minimum pain. All entry forms have been kept with the Investigator. The study was strictly voluntarily and a few potential study participants declined for fear of having their blood drawn from them.

CHAPTER FOUR: Results

Demographic characteristics of the hypertensive and normotensive groups

The study comprised 100 subjects who consented to participate.50 were known hypertension patients and 50 were healthy normotensive subjects who made the reference population.**Table 1** summarizes the main demographic data of the studied groups. There was no significant difference between hypertension patients and the normotensive subjects in relation to sex and haptoglobin polymorphisms in the studied groups. Age also had no statistical significance despite the hypertensive being older than the normotensive subjects.

Table 1. Demographic characteristics of the studied groups

Hypertensives					
Clinical variables		Hp 1-1	Hp 2-1	Hp 2-2	*P* value
Age		52.5 (±12.5)[1]	51.4 (±14.1)	57.1 (±9.79)	*p*[2]*0.17*
Sex	Males	13	2	7	
	Females	17	3	7	
Normotensives					
Clinical variables		Hp 1-1	Hp 2-1	Hp 2-2	
Age		34.2 (±6.7)	37.1 (±7.0)	31 (±7.1)	*p*[2] *0.178*
Sex	Males	4	4	6	
	Females	23	5	8	

Haptoglobin phenotypes distribution among the studied groups and associated risk

Table 2 shows haptoglobin phenotype 1-1 to be the most frequent in both study groups. The average prevalence of the Hp polymorphisms was found to be Hp 1-1 (58%), Hp 2-1 (14%) and Hp 2-2 (28%). In the hypertensive group 31 (62%) had Hp1-1 phenotype compared to 27 (54%) of the normotensives. However, there was no statistically

[1] Mean (±SD)
[2] *p* = Hp2-2 versus Hp 1-1

significant difference (p=0.238) between the two groups with regard to the occurrence of the Hp 1-1 phenotype. There was no significant difference also in the Hp 2-2 prevalence between the hypertensives and normotensives.

Table 2. Haptoglobin (Hp) phenotypes and Hp 1 allelic prevalence in the normotension and hypertension subjects

Population	Hp 1-1	Hp 2-1	Hp 2-2	Hp 1 prevalence
Normotension subjects (*N=50)*	27	9	14	54%
Hypertension subjects (*N=50)*	31	5	14	62%

The odds ratios (**Table 3**) indicate that none of the haptoglobin gene polymorphisms can be implicated as a risk factor in the development of hypertension. Hp 1-1 had an odds ratio of 1.45 but that was not statistically significant with the *p=0.32*. Hp 2-2 showed that it is neither protective nor a risk factor in the development of hypertension (OR = 1).

Table 3. ODDs ratio for the developing of hypertension with respect to haptoglobin (Hp) gene polymorphisms

Hypertension/Normotension	Hp 1-1	Hp 2-1	Hp 2-2
ODDs Ratio (95% CI)	1.45 (0.66-3.20)	0.51 (0.16-1.63)	1.0 (0.42-2.39)
P value	0.32	0.26	1.0

The haptoglobin serum levels in the hypertensive and normotensive groups with respect to the Hp phenotypes

Hp serum level did not differ significantly between phenotypes in the both groups, however Hp2-2 individual showed tendency toward lower levels. Table 4 shows the Hp serum levels with their statistical significance values.

Table 4. Hp serum levels in the studied groups according to the haptoglobin (Hp) gene polymorphisms

Hypertensives	Hp 1-1	Hp 2-1	Hp 2-2	*P* value
Serum levels[3]	1.80 (±0.48)	1.11 (±0.07)	0.73 (±0.25)	*0.282*
Normotensives				
Serum levels	1.70 (±0.49)	1.0 (±0.35)	0.69 (±0.20)	*0.282*

Figure 3 shows the descriptive frequencies of the haptoglobin serum levels in the two populations. Among the hypertensive subjects, the mean concentration of serum haptoglobin was 1.43 mg/mL and that of the normotensives was 1.11 mg/mL. The t-test showed that there was no significant difference between the two mean levels of serum haptoglobin as shown in the figure below.

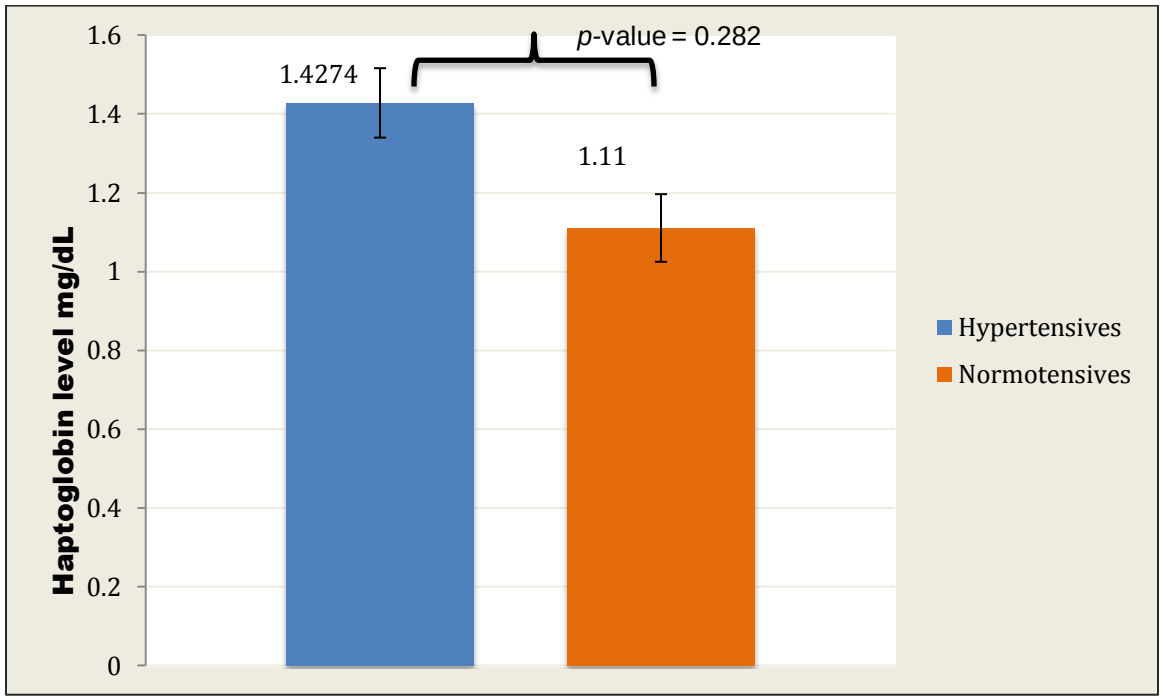


Figure 3. Hp Serum levels and hypertension

The haptoglobin serum concentrations were more elevated in the hypertensives in all the phenotypes than in the reference population. **Figure 4** shows the mean concentration for each phenotype in the two study groups. There were no statistical

[2] *p* = Hp2-2 versus Hp 1-1
[3] Serum levels in mg/mL

significant differences in the same phenotypes (e.g. Hp 1-1 versus Hp 1-1) between the hypertensives and the normotensives respectively.

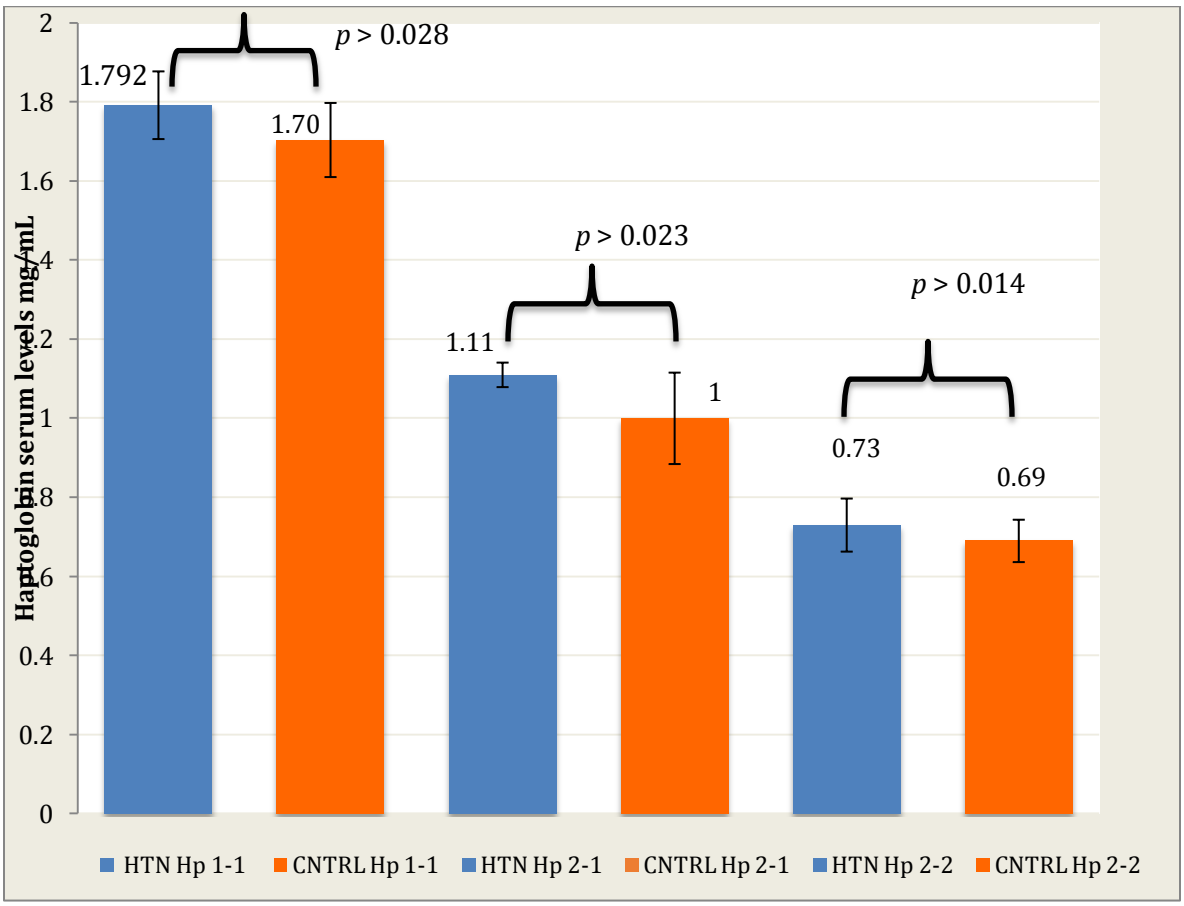


Figure 4. Hp Serum levels in the two study groups according to phenotype

The relationship of Body Mass Index with presence of Hp phenotypes in the studied groups

There were more individuals who were overweight among the hypertensives 33 (66%) compared to 26 (52%) in the normotensive study group (**Table 5**). Figure 5 illustrates this relationship. A correlation analysis was also done on the body mass index and Hp serum concentrations and phenotypes. Table 5 shows the BMI classifications of in the two groups according to their haptoglobin polymorphisms. 22 (67%) of those who were overweight among the hypertensives had Hp 1-1. This is compared to 13 (50%) who

were overweight in the normotensive group who also were Hp 1-1. However this did not have any statistical significance.

No difference was observed in the Hp phenotype 2-2 in both the hypertensive and normotensive groups as well.

Table 5. BMI distribution for normotension and hypertension subjects according to the haptoglobin phenotypes

Hypertensives				
BMI	Hp 1-1	Hp 2-1	Hp 2-2	*P*[4]value
Overweight[5]	22 (67%) [1.74 (±0.44)][6]	2 (6%) [1.04 (±0.03)]	9 (27%) [0.67 (±0.29)]	*p > 0.25*[7]
Normal weight	7 (50%) [1.82 (±0.24)]	2 (14%) [1.15 (±0.03)]	5 (36%) [0.82 (±0.12)]	
Underweight	2 (66%)	1 (33%)	0 (0%)	
Normotensives				
BMI	Hp 1-1	Hp 2-1	Hp 2-2	
Overweight	13 (50%) [1.76 (±0.34)]	1 (23%) [1.12 (±0.07)]	7 (27%) [0.74 (±0.22)]	*p > 0.33*
Normal weight	13 (57%) [1.64 (±0.62)]	3 (13%) [0.76 (±0.58)]	7 (30%) [0.64 (±0.19)]	
Underweight	0	0	0	

The relationship of BMI with serum levels of the phenotypes

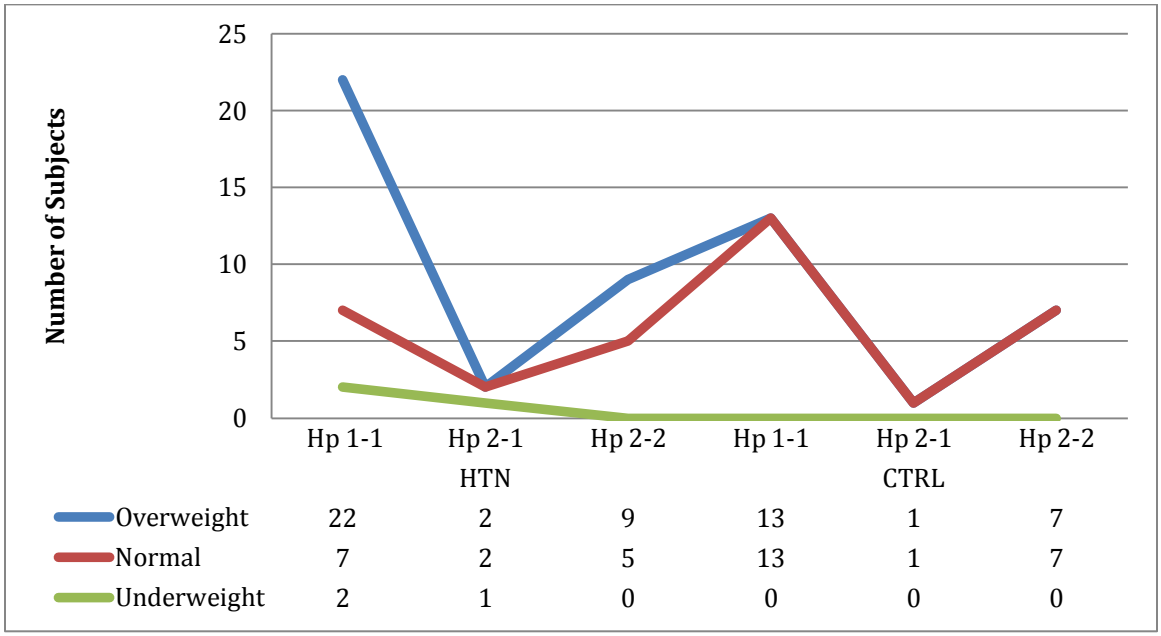
Thirty-three persons, (66%), of the hypertensives were classified as overweight and obese, while only 26 (52%) were classified as such among the normotensive individuals. In the hypertensive group the overweight and obese had the mean haptoglobin serum levels of 1.50 mg/mL (±0.69) compared to the normal-weights in the same hypertensive group of 1.43 mg/mL (±0.59). The normotensives had the mean haptoglobin serum levels of 1.36 mg/mL (±0.52) among the overweight and obese and 1.22 mg/mL (±0.70) among the normal-weight. However, there was no statistical significance in the differences between the two means in these groups. It was however

[4]*p* = Hp2-2 versus Hp 1-1
[5]Obese + overweight
[6]Mean Hp serum level (±SD)
[7]No Significance

notable that the haptoglobin serum levels were higher in the hypertensive group compared to the normotensives.Although there were more overweight and obese people among the hypertensives compared to the normotensives, there was no statistical difference between the two groups.

The results also showed that those who were overweight in both study groups had their haptoglobin serum levels higher than those who were normal weight in the same groups regardless of the haptoglobin phenotypes.

Figure 5.BMI vs Hp phenotypes in HTN and Control



CHAPTER FIVE: Discussion

Demographic data of haptoglobin phenotype and hypertension

Age and sex had no significant association with any of the haptoglobin phenotypes. The haptoglobin phenotypes were well distributed between the sexes and the various ages. Therefore it could not be implicated that a particular age group or sex are more prone to hypertension because they have a certain Hp phenotype. This is independent of the findings of Goma *et al,* (2011) which documented that the older ages and the male sex were associated with hypertension.

The prevalence of the haptoglobin polymorphisms in this study was found to be a ratio of Hp 1-1 58% and Hp 2-2 42%. The haptoglobin allelic prevalence was obtained from the general study population of both the hypertensives and normotensives. The prevalence was obtained after grouping the phenotypes in two categories of Hp 1-1 as group 1, and Hp 2-1 and Hp 2-2 as group 2. Despite the small sample size, the haptoglobin allelic prevalence compares well with what was observed in a study in Kenya where the Hp 1-1/Hp 2-2 ratio was 57%/43% (Herzog et al, 1970) and one in Ghana, which were Hp 1-1 52% and Hp 2-2 48% (Teye et al, 2006). Africa as a whole has an Hp 1-1 allele frequency of 56% with Hp 2-2 being 44% (Carter *et al, 2007*). In the American populations, the allele frequencies were observed as Hp1 54% and Hp2 46% Langlois et al, 1996). This then confirms that the equilibrium of the Hp1/Hp2 polymorphism is maybe broadly constant throughout the world (Vania et al, 2008).

Association between haptoglobin phenotypes and hypertension

This study has shown that there is no significant association between haptoglobin phenotypes and hypertension. None of the haptoglobin alleles were found to be a risk or protective factor of hypertension among the patients who were attending the medical clinic at the UTH in Lusaka, Zambia. This is unlike the findings of Hossein *et al*, (2004) which cited Hp 2-2 as a risk factor in the development of hypertension. Surya et al (1987) also observed that the haptoglobin patterns in essential hypertension and associated conditions had Hp 2-2 as a risk factor.

De Bacquer et al (2001) reported that in a cross sectional and case-control study, the Hp 2-2 has been associated with refractory arterial hypertension, myocardial infarct size, extension of coronary lesions and shorter graft survival time in CABG patients, and the prevalence of coronary and peripheral artery disease. Rather surprisingly, they identified the Hp 1-1 type as an independent risk factor for CHD death.

Yet another study linked the Hp 1-1 to hypertension as a causative factor. The possibility of a genetically mediated mechanism for the blood pressure response to alterations in sodium or water balance was suggested by Weinberger and his colleagues in their study. They observed that salt sensitivity was more likely to be observed in individuals with the homozygous haptoglobin 1-1 genotype than in those with the 2-2 genotype. And those individuals with the heterozygotic 2-1 genotype had responses that were intermediate between the other two groups. These findings were seen in both normotensive and hypertensive populations participating in two entirely different protocols for the assessment of salt responsiveness (Weinberger et al, 1987).

The findings of my study might look contradictory to those from earlier studies that implicate either Hp 2-2 or Hp 1-1 with increased risk for the development of hypertension and other cardiovascular diseases. But this maybe explained by the differing ethnic groups that present varying susceptibilities to hypertension posed by genetic factors in different parts of the world, especially in the Zambian context.

Serum haptoglobin levels and hypertension

Although there was no significant association established in the present findings between serum haptoglobin levels with their corresponding phenotypes and hypertension, yet there was a tendency for the mean serum levels of haptoglobin for the hypertension subjects to be higher than that of the normotension group. It was noted that regardless of particular phenotypes between the studied groups, the mean serum levels of haptoglobin were higher in hypertension compared to the normal reference group. However, because of lack of statistical significance in the differences in the mean

of the serum levels of haptoglobin in the subjects, it cannot be used as an indicator or diagnostic marker for hypertension.

In a study by Delanghe J et al (1999), observed that serum haptoglobin values were lower in Hp 2-2 patients compared to patients carrying another phenotype, but this is also the case for healthy subjects [1] and [6]. Other inflammatory parameters as well as serum lipids are not different according to Hp phenotype. My observations also showed proved similar findings. Those with HP 2-2 had lower serum levels of haptoglobin compared to those with Hp 1-1.

Association between BMI and haptoglobin phenotypes and hypertension

No significant relationship was observed between haptoglobin phenotypes and body mass index. Most hypertensive patients who were overweight had Hp 1-1 for their phenotype. It can therefore be hypothesised that those who have Hp 1-1 may be prone to being overweight and therefore to developing hypertension. Hp 2-2 on the other hand showed no variation in the two groups that were studied. The results showed that the distribution of the Hp 2-2 was the same regardless of the BMI classifications. It could not be proved that Hp 2-2 had a link to obesity and thereby the development of hypertension.

In recent years, obesity has been looked as a low-grade systemic inflammation condition (Das et al 2001). This view is supported by several lines of evidence, including the association of obesity with elevated serum levels of C-reactive protein (CRP), IL6, TNF, and leptin (Chiellini et al, 2004). Serum haptoglobin been one marker of inflammation explains why it was elevated in the overweight and obese subjects.

Endothelial dysfunction and haptoglobin phenotypes

The differences in the structure and sizes of Hp 1-1, Hp 2-1 and Hp 2-2 did not show any significance with regard to having hypertension or not. The study showed no significance in the association of haptoglobin phenotypes and hypertension. Literature studies however suggest that the Hp 2-2 has a size and structure that makes it unable

to form a stable complex of the Hb-Hp (Asleh et al, 2003). This results in the dysfunctional regulation of the NO bioavailabity and vascular homeostasis. This may cause endothelial dysfunction that is a risk factor for hypertension. The investigations of this study could not provide empirical evidence to support this link between haptoglobin phenotypes and hypertension.

Limitations of the study

The study was beset by two major limitations. One of the major limitations of this study was the poor phenotyping method used. This was constrained because of lack of adequate funding to carry out this study using more modern molecular technuiques to genotype and phenotype. Another limitation is that of a small sample size used.

CHAPTER SIX: Conclusion and Recommendations

The study observed that there is no haptoglobin phenotype that is a risk or protective factor for hypertension in individuals attending the medical clinic at the UTH, Lusaka. The study did not provide scientifically convincing associations between haptoglobin phenotypes and the increased risk of developing hypertension. Although conflicting studies exist which have findings contradictory to this one, the indigenous Zambian people do not seem to be at increased risk of developing hypertension because of having a particular haptoglobin phenotype. Again, the ethnic and genetic factors prevalent in our population may be responsible for this observation.

There is no significant association between serum levels of haptoglobin and hypertension. Serum haptoglobin as a parameter of inflammation was seen to be more elevated in those with hypertension than the normotensives. This was irrespective of the phenotype. Therefore the serum levels can be used as indicator of inflammation in hypertensive conditions and not as a diagnostic marker of hypertension.

No relationship was observed between haptoglobin phenotypes and body mass index. Those who are overweight and obese had higher levels of serum haptoglobin. But this again was irrespective of the phenotype. Going with the intriguing observation that obesity can be considered an inflammatory condition, it then provides possible explanations for the observed levels in the overweight and obese subjects. However, there was no particular haptoglobin which was linked to obesity which is a risk factor for the development of hypertension.

Recommendations

There is need to further study the susceptibilities that haptoglobin polymorphisms have on hypertension. This however must be done with a larger sample size using a longitudinal study approach. The Hp phenotyping must be done with more modern and good imprecision methods and techniques. In this way very conclusive association results could be obtained.

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APPENDIX

Information Sheet for Data Collection

I, Masauso M. Phiri, am conducting a research in fulfilment of the project requirements for the Master of Science in Pathology (Chemical Pathology) at the University of Zambia.

This study is aimed at investigating the association between the presence of haptoglobin phenotypes and hypertension in patients attending Outpatient Medical Clinic at the University Teaching Hospital (UTH), Lusaka. Haptoglobin phenotype 2-2 has been observed to be a risk factor in the development of hypertension in the Black population. Hence the information that has been obtained from this study proposes to examine this association and provide helpful information that will also add to the pool of knowledge on the genetic factors that can be implicated as risk factors in the development and poor prognosis of hypertension.

A blood sample was taken from the participant by venipuncture. When the needle is inserted to draw blood, some people feel moderate pain, while others feel only a prick or stinging sensation. Afterward, there may be some throbbing. There is very little risk involved with having blood taken from study participants. Veins and arteries vary in size from one person to another and from one side of the body to the other. Taking blood from some people may be more difficult than from others.

Other risks associated with having blood drawn are slight but may include:

- Excessive bleeding
- Fainting or feeling light-headed
- Hematoma (blood accumulating under the skin)
- Infection (a slight risk any time the skin is broken)

Should any of these study related injuries occur during the study, the attending medical doctor shall treat all such cases.

Hypertensive patients who were diagnosed during the study will be treated and advised accordingly.

Consent form

I understand the information concerning this research and explanation has been given to me in a language I understand better. I understand that I will be required to give of my blood for the testing of genes, which will be used in the investigation of an association between these genes and hypertension. I am aware that the process of drawing blood will be cause minimum pain and discomfort. I am further aware that all information will be treated as confidential and I will not be personally identified. I am therefore participating in this research on my own free will and I understand that I can withdraw at any time if I so wish.

Signature/Thumb impression: _____ Date: _____

Name: _____

Investigator's Signature: _____ Date: _____

If you have any further questions or clarification, please contact:

Mr. Masauso M. Phiri
University of Zambia
School of Medicine
Department of Pathology and Microbiology
University Teaching Hospital
Lusaka.
Email address: missiphiri@gmail.com
Mobile No.: +260 977930908

The Chairperson,
The University of Zambia Research Ethics Committee,
Ridgeway Campus,
P.O. Box 50110,
Lusaka, Zambia
Telephone: 260-1-256067
E-mail: unzarec@unza.zm