

# Haptoglobin Phenotypes and Hypertension in Indigenous Zambians at the University Teaching Hospital, Lusaka, Zambia

MM. Phiri,<sup>1</sup> T. Kaile,<sup>1</sup> FM. Goma<sup>2</sup>

<sup>1</sup>Department of Pathology and Microbiology, School of Medicine, University of Zambia

<sup>2</sup>Department of Physiological Sciences, School of Medicine, University of Zambia

## ABSTRACT

**Objectives:** The aim of the study was to investigate the association between presence of haptoglobin phenotypes and hypertension in indigenous Zambian patients attending outpatient medical clinic at the University Teaching Hospital in Lusaka, Zambia.

**Methodology:** The study was a descriptive, non-interventional, analytical, cross-sectional study involving haptoglobin quantification and phenotyping of hypertensive and normotensive subjects. The clinical characteristics and serum parameters of the study population were recorded and the haptoglobin phenotypes were determined.

**Results:** 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. There was no statistically significant difference between the two groups with regard to the occurrence of the Hp 1-1 phenotype in the studied groups and its relation to hypertension.

**Conclusion:** The study showed that there is no association between haptoglobin phenotype and hypertension.

## \*Corresponding Author:

Dr. Fastone M. Goma,  
Dept of Physiological Sciences,  
University of Zambia School of Medicine,  
P.O. Box 50110, Lusaka  
Email: gomafm@unza.zm

## INTRODUCTION

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. The prevalence of hypertension in sub-Saharan Africa ranges from 6% to 48%.<sup>1</sup> Despite recent advances in the understanding and treatment of hypertension, its prevalence continues to rise.<sup>2</sup> 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).<sup>3</sup> These prevalence estimates were higher than those in many other Southern African countries<sup>3</sup>.

Haptoglobin genes have been cited as risk factors in the development of hypertension.<sup>4</sup> 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. 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.<sup>5</sup> Haptoglobin polymorphism has been suggested as a candidate genetic marker in essential hypertension.<sup>6</sup>

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. The Haptoglobin-Haemoglobin (Hb-Hp)

complex is a NO scavenger. This has a role in regulating NO bioavailability and vascular homeostasis<sup>7</sup>. 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.<sup>8</sup>This reduces the risk of developing hypertension through endothelial dysfunction.

This association varies between populations<sup>9</sup> and therefore cannot be generalised. The study on which this paper reports on had the aim to investigate the association between presence of haptoglobin phenotypes and hypertension in patients attending outpatient medical clinic at the University Teaching Hospital, Lusaka.

## METHODOLOGY

### Study Design

The study was a descriptive, non-interventional, analytical, cross-sectional study involving haptoglobin quantification and phenotyping of hypertensive and normotensive subjects. The normotensive subjects acted as controls in the study.

### 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 53 Zambian men and women with already clinically diagnosed and known hypertension. Fifty-five 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 110 subjects were recruited for the study.

### Inclusion criteria

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

### Exclusion criteria

Less than 18 years old  
Diabetic and hypertensive subjects

### Data Collection

#### 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 backboard, 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 auscultator 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 hypertensive 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. The 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.

Haptoglobin phenotyping was done using the mean levels of the phenotypes as follows; Hp 1-1 ( $1.26 \pm 0.43$  g/L), Hp 2-1 ( $1.08 \pm 0.50$  g/L) and Hp 2-2 ( $0.84 \pm 0.42$  g/L). 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.

### 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.

### 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  $\pm$ SD. Student's (t) test was used for comparison of the serum levels between the two groups having quantitative normally distributed data. 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. P-value was considered statistically significant when it is less than 0.05. In the analysis, body mass Index (BMI) was categorized as  $<18.5$  kg/m<sup>2</sup> (lean), 18.5-24.9 kg/m<sup>2</sup> (normal), 25.0-29.9 kg/m<sup>2</sup> (over weight), and 30+ kg/m<sup>2</sup> (obese).

## RESULTS

### Demographic distribution in the hypertensive and normotensive groups

The results revealed that there was no difference between patients and the normotensives in relation to sex and age and haptoglobin polymorphisms in the studied groups.

### Haptoglobin phenotypes distribution among the studied groups and associated risk

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

The odds ratios (Table 1) 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 1.** 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

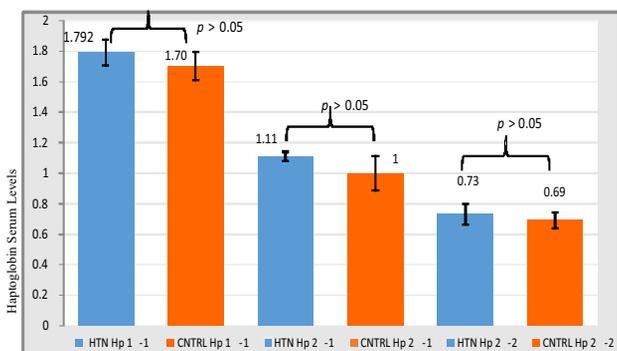
Hp serum level did not differ significantly between phenotypes in the both groups, however Hp2-2 individual showed tendency toward lower levels. Table 1 shows the Hp serum levels with their statistical significance values.

Among the hypertensive subjects, the mean concentration of serum haptoglobin was 1.43 mg/mL and that of the normotensives was 1.29 mg/mL. The t-test showed that there was no significant difference between the two mean levels of serum haptoglobin as the  $p$ -value was 0.282.

The haptoglobin serum concentrations were more elevated in the hypertensives in all the phenotypes than in

the reference population. **Figure 1** shows the mean concentration for each phenotype in the two study groups. There were no statistical significant differences in the same phenotypes (e.g. Hp 1-1 versus Hp 1-1) between the hypertensives and the normotensives respectively.

**Figure 1.** Hp Serum levels in the two study groups according to phenotype



**Table 2.** Demographic and clinical characteristics of the studied groups

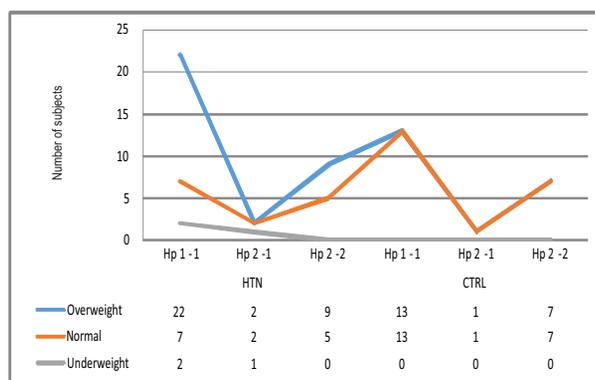
**The relationship of Body Mass Index with presence**

Hypertensives					
Clinical variables		Hp 1-1	Hp 2-1	Hp 2-2	P value
Age		52.5 (±12.5) <sup>1</sup>	51.4 (±14.1)	57.1 (±9.79)	$p^2 > 0.05$
Sex	Males	13	2	7	
	Females	17	3	7	
Serum levels <sup>3</sup>		1.80 (±0.48)	1.11 (±0.07)	0.73 (±0.25)	$p^2 < 0.05$
BMI	Overweight <sup>4</sup>	22 (67%) [1.74 (±0.44)] <sup>5</sup>	2 (6%) [1.04 (±0.03)]	9 (27%) [0.67 (±0.29)]	$p > 0.05^6$
	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					
Clinical variables		Hp 1-1	Hp 2-1	Hp 2-2	
Age		34.2 (±6.7)	37.1 (±7.0)	31 (±7.1)	$p^2 > 0.05$
Sex	Males	4	4	6	
	Females	23	5	8	
Serum levels		1.70 (±0.49)	1.0 (±0.35)	0.69 (±0.20)	$p^2 < 0.05$
BMI	Overweight	13 (50%) [1.76 (±0.34)]	1 (23%) [1.12 (±0.07)]	7 (27%) [0.74 (±0.22)]	$p > 0.05$
	Normal weight	13 (57%) [1.64 (±0.62)]	3 (13%) [0.76 (±0.58)]	7 (30%) [0.64 (±0.19)]	
	Underweight	0	0	0	

**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 2**). Figure 2 illustrates this relationship. Table 1 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 significance as the p value was greater than 0.05.

**Figure 2.** BMI vs Hp phenotypes in HTN and Control



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

**The relationship of BMI with serum levels of the phenotypes**

Thirty-three, (66%), of the hypertensives were classified as overweight (encompassing both the overweight and obese groups in the BMI classifications), while 26 (52%) were classified as such among the normotensive individuals. In the hypertensive group the overweight 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 ( $\pm 0.59$ ). The normotensives had the mean haptoglobin serum levels of 1.36 mg/mL ( $\pm 0.52$ ) among the overweight and 1.22 mg/mL ( $\pm 0.70$ ) among the normal-weight. There was no statistical significance in the differences between the two means in these groups. It was however notable that the haptoglobin serum levels were higher in the hypertensive group compared to the normotensives.

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.

## 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)<sup>3</sup> that documented that the older ages and the male sex are associated with hypertension.

### Haptoglobin phenotype distribution and prevalence

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. Despite the small sample size, the haptoglobin allelic prevalence compares well with what was observed in a study in Kenya. In that study it was observed that the Hp 1-1/Hp 2-2 ratio was 57%/43%. Another in Ghana found that Hp 1-1 was 52% and Hp 2-2 48%.<sup>10</sup> Africa as a whole has an Hp 1-1 allele frequency of 56% with Hp 2-2 being 44%.<sup>11</sup>

### 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 Hosein *et al*, (2004)<sup>5</sup> that cited Hp 2-2 as a risk factor in the development of hypertension. This maybe explained by the differing ethnic groups that present varying susceptibilities to hypertension posed by genetic factors<sup>9</sup>.

### 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 the mean serum levels of haptoglobin for the hypertension subjects was higher than that of the normotension 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.

### 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. This however could not be used as a link to predict that those who have Hp 1-1 are prone to be overweight and therefore develop 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.

## LIMITATIONS OF THE STUDY

One of the major limitations of this study is the poor phenotyping method used. This was constrained because of lack of adequate funding to carry out this study. Another limitation is that of a small sample size.

## CONCLUSION

The study shows that (i) there is no haptoglobin phenotype that is a risk or protective factor for hypertension in individuals attending the medical clinic at

the UTH, Lusaka. (ii) There is no significant association between serum levels and hypertension. (iii) There is no relationship between haptoglobin phenotypes and body mass index.

### RECOMMENDATION

There is need to study further the susceptibilities that haptoglobin polymorphisms has on hypertension. This however must be done with a larger sample size. The Hp phenotyping must be done with more modern and good imprecision methods and techniques. Only in this way can very conclusive and evidence based results be obtained.

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