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Interplay between nitric oxide (NO) and glucose 6-phosphate dehydrogenase (G6PD) activity in primary hypertension

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Essential hypertension is one of the most prevalent non-communicable diseases in sub-Saharan Africa and elsewhere in people of sub-Saharan origin. This study investigated the role of nitric oxide (NO) and glucose 6-phosphate dehydrogenase (G6PD) activity in aetiology of essential hypertension. An analytical cross-sectional design was applied to 89 essential hypertensive participants and 89 healthy normotensive participants, making a total of 178. Blood was collected for G6PD activity and serum levels of nitric oxide, glucose, creatinine, urea and electrolytes. Analysis of variance was employed to establish whether there was a difference in mean levels of NO between those that were G6PD deficient and those who were not. Lower NO levels were observed in those who were G6PD deficient though the difference was not statistically significant. A logistic regression was used to investigate the association of age, sex, NO levels, and G6PD deficiency with essential hypertension as the dependant variable. It was established that with an increase in NO levels there was less likelihood of developing hypertension (odd ratio (OR)=0.99), whereas individuals with impaired G6PD activity were 2.9 times more likely to develop hypertension than those with normal activity (OR=2.9). Our conclusion was that NO is important in prevention of hypertension through its vasodilator effect on arterioles.

Key words: Hypertension, nitric oxide (NO), glucose 6-phosphate dehydrogenase (G6PD), reactive oxygen species.

INTRODUCTION

Hypertension by definition is an increase in blood pressure above normal. If this is caused by an identifiable underlying factor, it is known as secondary hypertension. If no cause can be found, then it is primary hypertension and most hypertensive individuals belong to this category. Though no identifiable cause can be found for primary hypertension, there are always risk factors that contribute to its development (Wamala et al., 2009). Primary hypertension is a world-wide problem with variations in prevalence, with the lowest prevalence in rural India (3.4% in men and 6.8% in women) and the highest prevalence in Poland (68.9% in men and 72.5% in women) (Kearney et al., 2004). In fact hypertension is the most common cardiovascular disorder affecting

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approximately 1 billion people globally and accounts for approximately 7.1 million deaths annually (Brundtland, 2002). Primary hypertension is one of the most common non-communicable diseases in Zambia with high morbidity due to cardiovascular complications that result from untreated cases. Though no national surveys have been carried out, evidence coming from localized surveys and hospital statistics suggest that hypertension is a very prevalent medical condition. For instance a population based survey carried out in Lusaka Urban district of Zambia showed the prevalence of hypertension to be 34.8% (38.0% of males and 33.3% of females) (Goma et al., 2011). What is even more disturbing is the fact that many people with hypertension are not aware that they have the condition (France, 1999). Studies have shown that ethnicity, emotional stress, genetic factors, advancing age, obesity, alcohol consumption, level of education and inactivity are some of the risk factors leading to development of hypertension (Kulkarni et al., 1998; Anderson, 1999; Dominiczak et al., 2000; Vasan et al., 2002).

Untreated high blood pressure leads to serious complications like stroke, heart failure, myocardial infarction, renal insufficiency or failure, peripheral vascular disease, retinopathy, dementia, with high morbidity and mortality (Flack et al., 2003).

Among the many chemical messengers that seem to have a role in regulation of blood pressure is nitric oxide (NO) (Dominiczak and Bohr, 1995). NO is involved in a lot of physiological processes like immune responses, cell signalling, neurotransmission and many others (Rosselli et al., 1998). It is formed from L-arginine by endothelial NO synthase using reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor (Mehta et al., 2003). The main source of this NADPH is the pentose phosphate pathway, whose rate limiting step is catalysed by glucose 6-phosphate dehydrogenase (G6PD); hence, changes in activity of this enzyme can affect production of NADPH accordingly. Apart from NADPH acting as a cofactor in the synthesis of NO, it also provides reducing power for destruction of hydroxyl radicals that inactivate NO. 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 (Ignarro, 1989). Therefore, we decided to investigate if there was an association between NO levels and essential hypertension in Zambian participants. This study also went on to determine if there was a significant difference in NO levels between G6PD deficient individuals and those with normal G6PD activity.

MATERIALS AND METHODS

This study was conducted at the University Teaching Hospital (UTH), the largest national referral hospital in Zambia. It provides treatment services and medical check-ups for most of the population in Lusaka. An analytical cross-sectional study design was used. The target population included all the hypertensive patients coming to filter clinic to be attended to, and controls were mainly participants who were coming to the hospital with minor ailments or for medical check-ups. All participants had to undergo a thorough medical examination to rule out any chronic diseases or secondary causes of hypertension. Any client who was between 35 and 65 years inclusive and willing to participate was recruited. Those who were pregnant, obese, chronically ill or with secondary causes of hypertension were excluded. The systematic random sampling method was employed, where every 3rd person was selected for the study after a thorough medical examination by the medical doctor on duty. About 4 ml of venous blood was collected from the antecubital vein for the purposes of determining G6PD activity, blood levels of nitric oxide, glucose, creatinine, urea and electrolytes. Based on an expected prevalence of essential hypertension of 50% in the general population and 6% G6PD deficiency in normotensives, 89 participants were enrolled, giving a total of 178 participants.

NO levels were determined as described elsewhere (Moshage et al., 1995). Briefly, this assay determines NO based on the enzymatic conversion of nitrate to nitrite by nitratereductase. The reaction is followed by a colorimetric detection of nitrite as an azo dye product of the Griess reaction. G6PD activity was measured using the quantitative spectrophotometric method as outlined elsewhere (Lohr and Waller, 1958). In brief, the method relies on the fact that nicotinamideadenine dinucleotide phosphate ($NADP^+$) is reduced to NADPH by G6PD in the presence of glucose 6-phosphate. The rate of formation of NADPH is proportional to the activity of G6PD and is measured spectrophotometrically as in increase in the absorbance at 340 nm.

Statistics

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). Means and standard deviations were used to come up with the descriptive statistics for continuous variables. The continuous and categorical variables were compared using the unpaired student t-test and chi-square, respectively. In order to determine whether the mean of NO levels differed between those who were G6PD deficient and those who were not, analysis of variance (ANOVA) was employed.

Other variables that are believed to influence essential hypertension such as gender and age 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 rule out possible confounders. The odds ratios were used to establish the degree of association between NO and essential hypertension.

RESULTS

The study group consisted of 89 hypertensive patients with a mean age of 52.6 ± 10.4 which was greater than that in the control group (43.8 ± 9.0 years) and the difference was significant ($P < 0.0000$). NO levels in essential hypertensives were significantly lower than in normotensives ($p < 0.001$). Other variables whose means came out to be significant at 5% included serum glucose, creatinine and sodium levels all with p-values less than

Table 1. Descriptive statistics for essential hypertensive patients and control participants.

Parameter	Essential hypertensive patients (study group) (n=89)		Normotensive (control group) (n=89)		P-values (cases vs. controls)
	Mean	SD	Mean	SD	
Age (Years)	52.1	10.4	43.8	9.0	<0.000
NO (µM)	70.0	39.9	96.5	48.6	<0.001
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

Table 2. NO levels by G6PD ANOVA results.

NO levels by G6PD	ANOVA				
	Sum of squares	df	Mean square	F	Sig.
Between groups	1682.899	1	1682.899	0.785	0.377
Within groups	377109.489	176	2142.668		
Total	378792.388	177	-	-	-

Table 3. NO levels mean comparisons between those with G6PD deficiency and those with normal G6PD activity.

NO levels by G6PD	Mean	N	Standard deviation
Less than 10.01 U/g hemoglobin	75.30	23	37.886
Greater than 10.01 U/g hemoglobin	84.47	155	47.368
Total	83.29	178	46.261

0.05 (Table 1).

In order to test whether the mean of NO levels differed between those who are G6PD deficient and those who are not, ANOVA was employed and the results of the analysis are presented as shown in Table 2.

The results of the analysis of variance show that the mean of the dependent variable (NO levels) does not differ significantly (P-value = 0.377) between those with G6PD deficiency and those with normal G6PD activity. This is despite the comparison of means (Table 3) showing that those with G6PD activity greater than 10.01 U/g hemoglobin (Hb) have a higher value of NO level (84.47 µM) compared to those with G6PD deficiency (NO level of 75.30 µM) (Table 3).

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 presents the results for the unadjusted odds ratio for the bivariate logistic regression. The results indicate that only age and the levels of NO have a significant effect on hypertension at 5% significance level. The findings also indicated a significant association between NO 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 5 reveals that age, and G6PD deficiency all are positive and significantly associated with increased risk of hypertension, while NO is associated with reduced risk of essential hypertension. 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 NO levels was more likely to lead to decreased risk of having essential hypertension (OR = 0.99, CI = 0.98 - 0.99).

Table 4. Bivariate logistic regression with essential hypertension as the dependent variable with unadjusted odds ratios (Number of cases = 89; Number of controls = 89).

Variable		P-value	Unadjusted OR (95% CI)
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 (uM)	-	0.00	0.98 (0.98-0.99)

Table 5. Logistic regression for predictors of development of essential hypertension among 35-65 year olds screened at UTH.

Variable		Found with essential hypertension after screening		Unadjusted OR (95% CI)	Adjusted OR (95% CI)
		Yes [n (%)]	No [n (%)]		
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 (uM)	-	-	-	0.98 (0.98-0.99)	0.98 (0.97-0.99)

OR: Odds ratio; NO: nitric oxide; CI: confidence interval; G6PD: glucose-6-phosphate dehydrogenase.

DISCUSSION

Our findings in this study show that an increase in the NO is less likely to lead to development of essential hypertension based on the multivariate logistic regression where the odds ratio was established to be 0.99 (OR < 1). These findings corroborate what has been reported by others. NO levels were found to be significantly lower in women in prehypertensive phase of essential hypertension. In this case, lower NO correlated with increased systolic blood pressure (Gerasimovska-Kitanovska et al., 2005). In a case control study carried out in India, the findings suggested that the normal homeostasis of NO and adhesion molecules may play a significant role in the pathophysiology and hence, the risk of essential hypertension (Srivastava et al., 2006). In another study, it was found that dilator action of endothelium-derived NO contributed to the control of basal and stimulated regional blood flow in humans (Vallance et al., 1989). Impairment of production of NO might account for the abnormalities in vascular reactivity that characterise a wide variety of disease states. From this we concluded that NO is important in the maintenance of normal blood pressure and its increase may lead to less chances of developing hypertension. It can therefore be concluded from the study that there is an association between NO levels and essential hypertension, where an increase in the NO levels leads to reduced risk of developing essential hypertension.

Even though our results showed that NO levels were significantly lower in hypertensives than normotensives, there was no significant difference in mean levels of NO between G6PD deficient individuals and those with normal G6PD activity irrespective of blood pressure status. The probable mechanism for the interplay between NO and G6PD activity in primary hypertension may be that reduced G6PD activity forms less NADPH which eventually leads to decreased rate of NO synthesis. Reduced NO synthesis in turn minimizes vasodilator action on smooth muscle in arterioles. In addition, NADPH provides reducing power for removal of hydroxyl radicals which inactivate NO. Therefore, when NADPH is reduced due to inactivity of G6PD, more NO is inactivated predisposing the individual to hypertension as the vasodilator effect will have been curtailed.

It was also found out as expected, age to have a positive association with essential hypertension. This is supported by a study conducted on the prevalence of hypertension and it correlates in Lusaka Urban district of Zambia, where it was found that age and sex were

associated with hypertension (Goma et al., 2011). In our study, however, it was found out that sex had no association with essential hypertension [OR] = 0.79, [CI] = 0.39 - 1.60 (P = 0.6). Systolic blood pressure and pulse pressure increase with age mainly because of reduced elasticity (increased stiffness) of the large conduit arteries. Arteriosclerosis in these arteries results from collagen deposition and smooth-muscle cell hypertrophy, as well as thinning, fragmenting, and fracture of elastin fibers in the media (Oparil et al., 2003). Endothelial dysfunction due to age may reduce synthesis of NO which in turn could reduce arterial compliance resulting in increased blood pressure (Safar, 1999).

It is worth noting that the liver is one of the organs where the pentose phosphate pathway, the main source of NADPH, is very active. In view of the fact that obesity, one of the risk factors for development of essential hypertension, is associated with non-alcoholic fatty liver disease patients should have been screened by ultra-sound for the presence of hepatic steatosis (Tarantino et al., 2012), but this could not be done due to limited facilities. This is important because steatosis is asso-ciated with a high risk of developing type 2 diabetes mellitus, dyslipidemia (high plasma TG and/or low plasma HDL-cholesterol concentrations), and hypertension (Adams et al., 2005). In addition, levels of serum alanine amino transferase and γ–glutamyltransferase should have been determined as it has been shown that high levels γ–glutamyltransferase is associated with hyperten-sion (Tarantino et al., 2012). Steatosis may predispose to hypertension probably because the pathological change associated with the condition impairs the pentose phosphate pathway leading to reduction in NADPH formation.

Our conclusion was that NO is important in prevention of hypertension through its vasodilator effect on arterioles. It remains to be elucidated whether essential hypertension is caused by a defect in endothelial NO 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 supple-ments, which upon metabolism release NO would be given in essential hypertension. Additional studies will be required to determine the NO levels in hypertensives with normal and low G6PD activity; and in normotensives with normal and low G6PD activity.

Conflict of Interests

The author(s) have not declared any conflict of interests

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