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Endothelial Nitric Oxide Synthase (eNOS) Levels in Hypertensive Black Zambian Patients

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Abstract

Endothelial nitric oxide synthase (eNOS) is a very important vasodilator and its levels in the blood are maintained to allow for proper perfusion and easy blood flow. Low plasma levels of eNOS might lead to hypertension. We carried out a study to ascertain eNOS levels in indigenous black Zambians with hypertension. An analytical cross sectional study was undertaken in 82 (72 female and 10 male) black Zambians. The age range was 53.2±1.8 years in the hypertensives and 47.5±1.5 years in the non hypertensives. Plasma endothelial nitric oxide synthase was determined using the NeoBioLab® Human Endothelial Nitric Oxide Synthase 3 ELISA Kit; a sandwich immunoassay for the quantitative measurement of human endothelial nitric oxide synthase in cell culture fluid, body fluid, tissue homogenate, serum or blood plasma. The eNOS ELISA test was performed in the KS-HHV8 Diagnostic and Research Laboratory in the University Teaching Hospital (UTH). ELISA plates for plasma eNOS were read on the microwell reader available in the KS-HHV8 Diagnostic and Research Laboratory in the UTH. Our results showed that eNOS was 1.5±0.2 ng/ml in hypertensives and 1.2± 0.1ng/ml in non hypertensive participants. However there was no significant difference in eNOS levels between hypertensive and non-hypertensive participants (P=0.197). The study showed that there was no difference in mean eNOS concentration between hypertensive and non-hypertensive participants. This could suggest that other factors other than eNOS could be responsible for the development of hypertension in indigenous black Zambians.

Key words: eNOS, Hypertension, ELISA, normotensive, Zambians

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Introduction

Nitric oxide (produced by endothelial nitric oxide synthase) is a small (30 kDa) gaseous and lipophilic molecule, key properties that enable it to function as a juxtacrine mediator of cell signalling processes that modulate blood vessel relaxation and help in maintaining normal blood pressure (Kerwin et al, 1995).

The endothelium, a single-layered continuous cell sheet lining the luminal vessel wall, separates the intravascular (blood) from the interstitial compartment and the vascular smooth muscle. Based on cell count ($6x10^{13}$), mass (1.5kg), and surface area ($1000m^2$) the endothelium is an autonomous organ (Thomas et al, 2008). Though for a long time regarded as a passive barrier for blood cells and macro solutes, this view completely changed with the advent of endothelial autacoids like prostacyclin (PGI_2) (moncada et al, 1978; Thomas et al, 2008), and nitric oxide (NO) (Palmer et al, 1987), as well as with the discovery of integrins and other surface signals (Stupak and Cheresh, 2004).

It is now evident that the endothelium is not only at the cross-bridges of communication between blood and tissue of surrounding cells by a plethora of signalling routes. One of the prominent communication lines is established by the so called L-arginine, NO cyclic Guanosine monophosphate (cGMP) pathway (Busse and Fleming, 1998). This signalling cascade starts with endothelial nitric oxide synthase (eNOS), which generates NO and L-

citrulline from L-arginine and oxygen gas in response to receptor-dependent agonists (bradykinin, acetylcholine, Adenosine triphosphate and physico chemical stimuli like stretch) (Fleming and Busse, 2003).

NO produced in the endothelial cell diffuses to the lumen where it is captured by erythrocytes and transferred into muscle cells where it induces relaxation, eliciting vasodilation. In this mechanism, guanosine 3'5'- cyclic mono phosphate (cGMP), formed from guanosine 5' triphosphate by the action of guanylate cyclase, is activated by NO cGMP modulates the myosin light chain (MLC) phosphatase positively and MLC kinase negatively, resulting in the dephosphorylation of MLC with subsequent muscle relaxation (Ignarro, 2002). Therefore, low eNOS causes low NO leading to impaired vascular relaxation and increasing peripheral resistance thereby elevating the blood pressure.

Materials and Methods

***Selection of participants and specimen collection*:** Blood was collected with informed consent from hypertensive black individuals reporting to the medical clinic 5 at the University Teaching Hospital, Lusaka, Zambia. Participants (Study controls) were recruited from healthy individuals who came for medical check-ups at high cost clinic in the University Teaching Hospital. The study controls were also required to give informed consent. Blood samples were collected from the research participants via veno-puncture in a 5ml syringe using 21G bore size needles. This study was cleared by University of Zambia Biomedical Research Ethics Committee reference 012-05-14.

***Specimen Preparation and Storage* –** In the laboratory, each specimen serial number was recorded onto a compilation summary sheet. Thereafter the blood specimen was centrifuged at 3000 revolutions per minute 3000 (rpm) in order to separate the plasma (supernatant) from the blood cellular components (sediment). Only supernatant (plasma) was then meticulously collected from the lithium heparin vacutainers using pipettes and transferred to 2ml plastic cryovial containers with sealable screw caps which was stored in a freezer at –80^{o}c until the specimens were analysed in a batch.

eNOS Estimation – Plasma eNOS concentration was determined using the NeoBioLab® Human Endothelial Nitric Oxide Synthase 3 ELISA Kit; a sandwich immuno assay for the quantitative measurement of human endothelial nitric oxide synthase in cell culture fluid, body fluid, tissue homogenate, serum or blood plasma (manufactured and supplied by NeoBioLab®, 395 W Cummings Park. Woburn, MA 01801, USA) according to the manufacturers protocol. This assay employed an antibody specific for human endothelial nitric oxide synthase coated on a 96-well plate. ELISA plates were read using the Versa Max Plus Rom V1.23 ELISA Plate reader.

Results

This study found that the mean eNOS concentration in the hypertensive participants was (1.5±0.2 ng/ml) and (1.2±0.1ng/ml), in the non-hypertensive group P=0.197 showing that there was no difference in the mean eNOS concentration between the two groups.

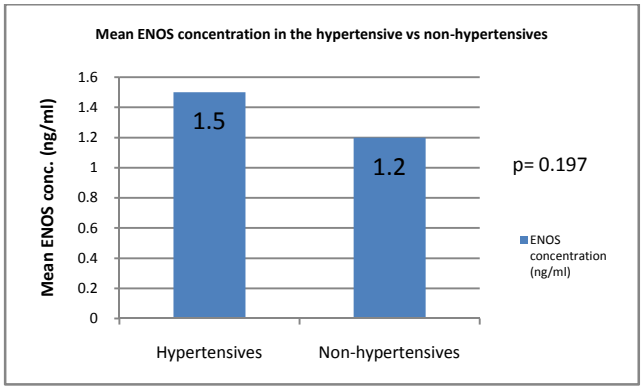

Figure 1: eNOS mean concentration in hypertensive vs non-hypertensives.

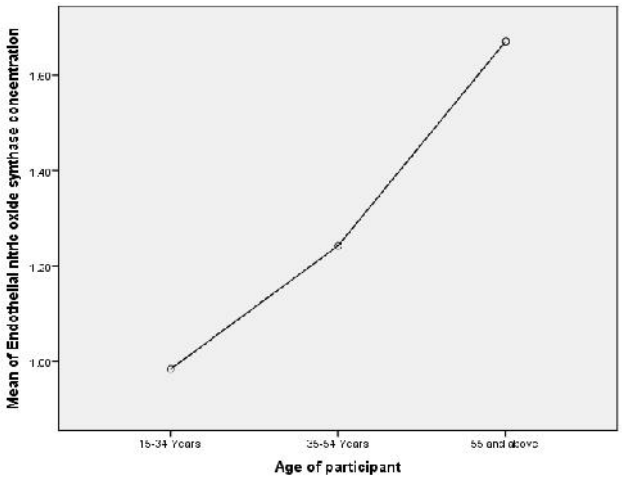

Figure 2: The figure showed that eNOS was increasing with increase in age. It presented with development of hypertension in younger age group suggesting the increase in hypertension could be secondary to other factors other than reduced eNOS levels.

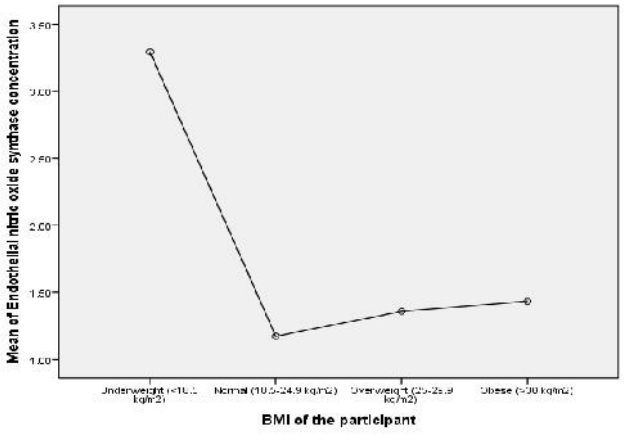

Figure 3: Relationship of BMI categories vs Mean eNOS concentration. The figure showed that a high eNOS in underweight was reducing with an increase in BMI (where n=underweight 2, normal weight 28, overweight 24 and obese 28) to reach optimum levels comparable to participants with normal BMI; suggesting again that, other factors are more predominant in development of hypertension in indigenous black Zambians than eNOS.

Discussion

Nitric oxide (produced by eNOS) is a small (30kDa) gaseous and lipophilic molecule, Key properties that enabled it to function as a juxtacrine mediator of cell signaling processes that modulated blood vessel relaxation and helped in maintaining normal blood pressure (Kerwin *et al*, 1995).

Our study found that the mean endothelial nitric oxide synthase concentration was slightly higher in the hypertensive group (1.5 ± 0.2 ng/ml) compared with the non-hypertensive group (1.2 ± 0.1 ng/ml) but was not statistically significant, t(82)=1.352, p=0.197 (Fig. 1). However, we expected to find higher concentrations of endothelial nitric oxide synthase in the normotensive healthy participants than in the hypertensive group. Other studies found that patients with essential hypertension had low levels of eNOS (Heller, 1996), which was the enzyme for synthesis of NO in vascular cells (Auggard, 1994; Bredt and Synder, 1994; Henrich, 1991; Murod, 1999). These findings were in contrast to those by Chandra *et al*, (2003) who reported that essential hypertensive patients had lower NO concentration levels than normotensive healthy subjects (18.59 ± 7.46 compared with 33.04 ± 2.8 mMol/l respectively; p<0.001) which was an estimate of the activity of endothelial nitric oxide synthase. Arora *et al*, (2009) reported that nitrite levels, a metabolite of NO was lower in the hypertensive group than the non-hypertensive group (4.0 ± 1.7 compared with 6.7 ± 3.2 um respectively; p<0.001). Our indigenous black Zambians did not show any significant difference in mean eNOS levels (fig. 1). This might suggest that the development of hypertension in our target population might follow an alternative pathway other than increased vascular tone or increased peripheral resistance. Alternatively, the non-significant differences for endothelial nitric oxide synthase might have been due to small sample size involved in this study.

A number of factors are known to affect the concentration of endothelial nitric oxide synthase in plasma. It has been shown that angiotensin-converting-enzyme inhibitors and angiotensin II receptor type 1 antagonists increase NO production not only in nephrogenic but also in essential hypertension (Slaninka-Miceska *et al*, 2003; Yavuz *et al*, 2003). The short acting beta-blockers inhibit NO production and decrease insulin sensitivity, while long-lasting beta-blockers and notably hybride beta-blockers such as carvedilol (Kalinowski *et al*, 2003) or nebivolol (Fratta Pasini *et al*, 2005) increase NO production. Calcium antagonists like dihydropyridines a benzodiazepines increased it, while phenylalkylamines do not influence NO production (Ding and Vaziri, 2000). Most of our hypertensive participants have been on one or a combination of these drugs. Thereby, confounding the findings on the plasma concentrations of endothelial nitric oxide synthase, in our study.

Our study also revealed that the mean of endothelial nitric oxide synthase concentration was increasing with age but it was not statistically significant (p=0.206). These findings were similar to those by Alusik *et al*, (2008) in which a group of apparently healthy elderly individuals aged over 80 had shown to have increased plasma levels of nitrates (29.3 ± 12.0 umol/l) compared with controls (21.38 ± 5.81 umol/l); however, the difference was not statistically significant. In addition to dietary and renal factors, the increased levels in the study by Alusik *et al* may have been due to enhanced NO production by inducible nitric oxide synthase in asymptomatic inflammation. Our study showed that the mean of endothelial nitric oxide sythase concentration was high in those who were underweight, this may mean an increase in eNOS per BMI, which could be normal per given BMI unit. Mean eNOS levels in overweight and obese were similar to those with hypertension although there was no statistically significant (p=0.078) difference. There is need for a bigger study to be done and also the genetic profile of the eNOS gene should be done

as well to allow for better and comprehensive understanding.

Conclusion

The study showed that there was no difference in mean eNOS concentration between hypertensive patients and non-hypertensive participants. This could suggest that other factors other than eNOS could be responsible for the development of hypertension in indigenous black Zambians.

Conflict of interest: None declared.

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References

1. Kerwin JF Jr, Lancaster JR Jr, Feldman PL. Nitric Oxide. A new paradigm for second messengers. J Med Chem. 1995; 38: 4343-4362.
2. Thomas M, Christop S, Felix P, Ascan W, Eberhard S. Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. Annals of Medicine. 2008; 1:1-17.
3. Moncada S, Korbut R, Buding S, Vane JR. Prostacyclin is a circulating hormone. Nature. 1978; 273: 767-768.
4. Palmer RM, Ferrige AG, Moncada S. Nitric Oxide release accounts for the biological activity of endothelium-derived relaxing factor. Nature. 1987; 327: 524-526.
5. Stupack DG, Cheresh DA. Integrin's and angiogenesis. Cu Top Dev Biol. 2004; 64:207-238.
6. Busse R, Flemming I. Regulation of NO synthesis in endothelial cells. Kidney Blood Press Res. 1998; 21:264-266.
7. Flemming I. Busse R. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. Am. J Pysiol Regul Integr Comp Physiol, 2003; 284:R1-12.
8. Ignarro LJ. Nitric oxide as a unique signalling molecule in the vascular system: A historical overview. J. Physiol, Pharmacol, 2002; 53:503-514
9. Mahmud A, Freely J. Adiponectin and arterial stiffness. Am J Hyperterns. 2005; 18:1543-1548.
10. Han SH, Quon MJ, Kim J, Koh KK. Adiponectin and cardiovascular disease, a response to therapeutic intervention. J AM Coll Cardiol. 2007; 49:531-538.
11. Giuseppe S, Matteo P. A novel link between obesity and hypertension. Hypertension. 2007; 49: 1217-1219.
12. Heller H. Hypertension, the endothelium, and the Pathogenesis of Chronic Vascular disease. Kidney and blood pressure research. 1996; 19:166-171.
13. Auggard E. Nitric Oxide: mediator, murder and medicine. Lancet. 1994; 343:1199-1206.
14. Bredt D, Snyder SH. Nitric oxide a Physiologic Messenger molecule. Annu Rev Biochem. 1994; 3:175-195.
15. Henrich W. The endothelium-a key regulator to vascular tone. American Journal of Medical Science. 1991; 302:319-328.
16. Murod F. Discovery of some of the Biological Effects of Nitric oxide and its Role in Cell Signalling. Nobel Lecture. Bioscience Reports. 1999; 19:133-153.
17. Chandra M, Maurya DR. Kumer S, Basara H, Ghatak A, Tekwani BL. Reversible suppression of Nitric oxide System in essential hypertension. Indian Journal of clinical Biochemistry. 2003; 18(2): 150-153.
18. Arora S, Das N, Srivastav K. Nitric Oxide and eNOS Gene in Essential Hypertension. International Journal of Collaborative Research on Internal Medicine and Public Health. 2009; 1 (2): 56-71.
19. Slaninka – Miceska M, Bogdanska J, Korneti P, Kostova E, Jovanovska E, Petrov S. Effect of angiotensin II type 1 (ATI) receptor antagonist on the endothelial dysfunction in spontaneously hypertensive ratio correlation with the nitric oxide system. Bratisi Lek Listy, 2003; 104: 342-346.
20. Yavuz D, Koc M, Topra KA, Akpinar I, Velioglu A, Deyneli O, Haklar G, Alkalin S. Effects of ACE inhibition and ATI-receptor antagonism on endothelial function and insulin sensitivity in essential hypertension. J Renin Augiotension Aldosterone Syst. 2003; 3:197-203.
21. Kalinowski L, Dobrucki: LW, Szczepansskan Konkel M, Jankowski M, Martyniel L, Angielski S, Malinski T. Third generation beta-blockers stimulate nitric oxide release from endothelial cells through ATP efflux- a novel mechanism for anti-hypertensive action. Circulation. 2003; 107: 2747-2752.
22. Fratta Pasini A, Garbin U, Nava M C, Stranieri C, Davoli A, Sawaura T, Cacio VL, Cominacini L. Nebivolol decreases oxidative stress in essential hypertensive patients and increases nitric oxide by reducing its oxidative inactivation. J Hypertens. 2005; 23:589-596.
23. Ding Y, Vaziri ND. Nifedine and diltiazem but not verapamil up-regulate endothelial nitric oxide synthase expression. J. Pharmacol. Exp. Ther. 2000; 292: 606-609.
24. Alusik S, Jedlickova V, Paluch T, Zecova S. Plasma levels of nirite/nitrate and inflammation markers in elderly individuals. Bratisl Lek Listy. 2008; 109(7): 289-292.