Original Research Article

Serum nitric oxide and malondialdehyde in a hypertensive population in Sokoto, Nigeria

Yale B. M.*, Yeldu M. H.

Department of Chemical Pathology, Faculty of Medical Laboratory Sciences, Usman Danfodiyo University, Sokoto, Nigeria

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*Correspondence:
Dr. Yale B. M.,
E-mail: yalebala@gmail.com

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ABSTRACT

Background: Hypertension is recognized as most common cardiovascular disorder and a leading cause of morbidity and mortality worldwide. Endothelial dysfunction, which is associated with impaired nitric oxide is an important risk factor for both hypertension and cardiovascular diseases. There is abnormal lipid peroxidation which suggested that oxidative stress is important in the pathogenesis of hypertension. This study assessed serum levels of nitric oxide and malondialdehyde in hypertensive population in Sokoto-Nigeria.

Methods: A total of 474 subjects who are within the age range of 25 to 76 years, including 316 hypertensive patients and 158 age- and sex- matched normotensive subjects were included in this study. Clinical and anthropometric parameters, nitric oxide and malondialdehyde were measured using standard techniques.

Results: The result indicated that, mean systolic blood pressure (SBP) was significantly (p<0.001) higher in hypertensive patients (166.00±1.39mmHg) than controls (124.97±0.95 mmHg) similarly the mean BMI was significantly (p<0.001) higher in hypertensive patients (27.13±0.31 Kg/m²) than controls (23.54±0.12Kg/m²). Mean serum malondialdehyde (MDA) was significantly (p<0.001) higher in hypertensive patients (3.62±0.07µmol/L) as compared to controls (1.97±0.03µmol/L), while serum nitric oxide (NO) was significantly (p=0.009) lower among hypertensive patients (7.12±0.14µmol/L) than controls (15.26±0.15µmol/L).

Conclusions: Hypertension is a complex disorder that is strongly associated with other risk factors for cardiovascular disease. The aetiology of the association between impaired NO bioactivity, increase MDA and hypertension has not been fully elucidated. Further clarification of the role of impaired NO bioactivity and increased MDA level in hypertension could have important implications for the management of hypertension.

Keywords: Hypertension, Malondialdehyde, Nitric oxide

INTRODUCTION

The global burden of hypertension is an important and increasing health problem worldwide and that awareness and control of hypertension vary considerably.1 Hypertension has been recognized as the most common cardiovascular disorder and a leading cause of morbidity and mortality in both developed and developing countries.2 It has been recognized as one of ten leading reported causes of death with about 4% of such deaths due to hypertensive complications.2 The reported prevalence of hypertension varies around the world, with the lowest prevalence in rural India (3.4% in men and 6.8% in women) and highest in Poland (68.9% in men and 72.5% in women).1

In sub-Saharan Africa, the prevalence is reported as 32.6%.3 The prevalence in Nigeria varied from 11% to
45%. In Sokoto, the reported prevalence of hypertension is 24.8%.

Attenuated nitric oxide (NO) bioavailability, the main characteristic of endothelial dysfunction is present in arterial hypertension. Hypertensive subjects have increased generation of reactive oxygen species (ROS) which scavenge NO, thereby reducing its bioavailability.

It was shown in animal models that deletion of the endothelial nitric oxide synthase (eNOS) gene as well as chronic inhibition of nitric oxide synthase (NOS) with N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME) leads to the development of arterial hypertension. L-NAME infusion also induces endothelial dysfunction in humans, as does the NO\textsuperscript{-} inhibitor N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA).

Data concerning the effect of antihypertensive treatment with \( \beta \)-blockers on endothelial function depend on the specific \( \beta \)-blocker; the beneficial effect of a NO-releasing \( \beta \)-blocker, nebivolol on endothelial function results from the increase in NO and not from the \( \beta \)-blocking effects of the drug.

Angiotensin-converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) improve endothelial function partly independent of arterial pressure reduction, and calcium antagonists (dihydropyridine-like agents in particular) improve endothelial dysfunction, accompanied by a simultaneous improvement in several markers of oxidative stress.

These antioxidant actions are particularly important because oxidative stress and the resulting scavenging of NO by excessive ROS is believed to be a major cause of impaired NO bioactivity, leading to oxidation of biologic macromolecules including lipids, deoxyribonucleic acid (DNA), protein and carbohydrates, vascular inflammation, and the development of atherosclerosis and cardiovascular diseases (CVD).

Malondialdehyde (MDA) has been reported to be induced in various conditions and chronic disease states such as hypertension, smoking, hepatitis C infection and human immunodeficiency virus seropositive children and diabetic mellitus (DM). MDA is an aldehyde considered to be the terminal compound and the most important marker for monitoring lipid peroxidation and oxidative damage induced by ROS which is strongly associated with development of serious diseases. There is growing interest in lipid peroxidation and its relationships to human atherosclerosis and cardiovascular risk factors, including deficiency of antioxidants.

The term oxidative stress refers to conditions under which excessive production of ROS, possibly triggered by cardiovascular risk factors such as hypertension, smoking, obesity and dyslipidaemia, overcomes antioxidant defence mechanisms, such as NO bioactivity, leading to oxidation of biologic macromolecules including lipids, DNA, protein and carbohydrates, vascular inflammation and the development of atherosclerosis and CVD.

**METHODS**

This study was carried out at Usmanu Danfodiyo University Teaching Hospital and Specialist Hospital, Sokoto-Nigeria. Ethical clearance for the study was obtained from both institutions and an informed consent was obtained from each subject prior to the commencement of the study. A total of 474 subjects were recruited for the study.

These consisted of 316 diagnosed adult hypertensive patients and 158 age and sex matched normotensive subjects (controls).

The hypertensive patients were recruited from Medical and General Outpatient Departments and those admitted into Male and Female Medical Wards of Specialist Hospital and Usmanu Danfodiyo University Teaching Hospital, Sokoto, while the controls were recruited from a population of neighbouring community. Blood pressure (BP) measurement was carried out by the Consultant Physician using manual BP apparatus while anthropometric parameters were measured using standard techniques.

From each selected subject, 3ml of venous blood sample was collected using a sterile disposable syringe and needle and allowed to clot at room temperature after which it was centrifuged at 3000rpm for 5minutes to obtain a clear unhaemolzyed serum. The sera were harvested and placed in another plain tube and rapidly stored at -20°C until the time for analysis. Serum concentrations of NO were estimated by the method of Schmidt, using assay kit obtained from Enzo Life Sciences, USA. Serum concentrations of MDA were estimated by the chemical method of Shah and Walkers, using chemical reagents obtained from Loba Cheme Limited Mumbai, India.

**Statistical analysis**

The data generated were analyzed using statistical package for social sciences (SPSS) version 20. The results were analyzed and expressed as mean plus/minus standard error of the mean (Mean±SEM). The results of both the clinical and biochemical parameters obtained from hypertensive patients were compared with those of controls using paired two-tailed student’s t-test for matched samples. The p value less than 0.05 (p<0.05) was considered to be significant.

**RESULTS**

Table 1 shows the age and gender distribution of hypertensive patients and controls in their different age
classes. A total of 316 hypertensive patients were recruited (155 males and 161 females). A total of 158 apparently healthy and normotensive subjects were recruited (74 males and 84 females) as controls. Most of the subjects were within the age range of 41-56 years.

The results of the demographic characteristics of the study population are shown in Table 2. The results showed that most (92.8%) of the subjects were married, they were predominantly Hausas (85.9%) and some (39.5%) had no formal education.

Table 1: Age (years) and gender distribution of hypertensive patients and controls.

| Age class (years) | Hypertensive patients | Controls | Total |
|-------------------|-----------------------|----------|-------|
|                   | M (n=155) F (n=161)   | M (n=74) F (n=84) |       |
| 25-40             | 22 25                 | 12 16     | 75    |
| 41-56             | 57 80                 | 31 37     | 205   |
| 57-72             | 65 54                 | 28 28     | 175   |
| ≥73               | 11 2                  | 3 3       | 19    |
| Total             | 155 161               | 74 84     | 474   |

Table 2: Demographic characteristics of the study population.

| Characteristics     | No. of subjects (N=474) | Percentage (100%) |
|---------------------|-------------------------|-------------------|
| Marital Status      |                         |                   |
| Married             | 440                     | 92.83             |
| Single              | 21                      | 4.43              |
| Widowed             | 13                      | 2.74              |
| Tribe               |                         |                   |
| Hausa               | 407                     | 85.86             |
| Yoruba              | 22                      | 4.64              |
| Igbo                | 7                       | 1.48              |
| Others              | 38                      | 8.02              |
| Occupation          |                         |                   |
| Civil servants      | 151                     | 31.86             |
| Business men/women  | 29                      | 6.12              |
| Artisan             | 21                      | 4.43              |
| Students            | 21                      | 4.43              |
| Others              | 252                     | 53.16             |
| Level of education  |                         |                   |
| Tertiary            | 147                     | 31.01             |
| Secondary           | 57                      | 12.02             |
| Primary             | 83                      | 17.51             |
| No formal education | 187                     | 39.46             |

Values are distribution of subjects with percentages, N= number of subjects.

Table 3 showed the BP and anthropometric characteristics of the study population. The mean SBP, DBP, Weight, BMI and Waist circumference were significantly (p<0.001) higher in hypertensive patients than controls. The mean height in hypertensive patients were not significantly different (p= 0.117) from those of controls.

The mean serum levels of MDA and NO in hypertensive patients and controls are shown in Table 4. The mean serum MDA was significantly (p<0.001) higher, while that of NO was significantly (p=0.009) lower among hypertensive patients than controls. There was a significant positive correlation between age and serum MDA in hypertensive patients (r=0.190, p=0.001) as illustrated in Figure 1.

Likewise, a scatter plot (Figure 2) indicates a significant positive correlation between BMI and MDA in hypertensive patients (r=0.248, p<0.001).

Table 3: Blood pressure and anthropometric values of the study population.

| Variable          | Patients n= (316) | Controls n= (158) | p-value |
|-------------------|------------------|-------------------|---------|
| SBP (mmHg)        | 166.00±1.39      | 124.97±0.95       | <0.001  |
| DBP (mmHg)        | 98.44±0.74       | 81.57±0.27        | <0.001  |
| Weight (Kg)       | 71.76±0.75       | 63.51±0.46        | <0.001  |
| Height (m)        | 1.63±0.00        | 1.64±0.00         | 0.117   |
| BMI (Kg/m²)       | 27.13±0.31       | 23.54±0.12        | <0.001  |
| WC (cm)           | 92.89±0.75       | 84.42±0.35        | < 0.001 |
| Age (years)       | 53.68±0.68       | 52.97±0.95        | 0.862   |

Values are mean±SEM, n= number of subjects, SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = body mass index, WC = waist circumference

Table 4: Serum concentrations of malondialdehyde and nitric oxide among hypertensive patients and controls.

| Parameters         | Patients n= (316) | Controls n= (158) | p-value |
|--------------------|------------------|-------------------|---------|
| Malondialdehyde (µmol/L) | 3.62±0.07       | 1.97±0.03         | <0.001  |
| Nitric oxide (µmol/L)   | 7.12±0.14       | 15.26±0.15        | 0.009   |

Values are mean±SEM, n= number of subjects’ p<0.05 is considered significant.
DISCUSSION

The reduced serum level of NO in hypertensive patients was reported.\textsuperscript{16,17} Decrease synthesis and enhanced degradation of NO could be responsible. Oxidative stress resulting from a disturbance in the pro-oxidant-antioxidant balance in favour of the former may lead to scavenging of NO by ROS, possibly triggered by cardiovascular risk factors such as hypertension, smoking and obesity among others. Altered balance between NO and ROS could lead to impaired bioavailability of NO, resulting in decreased endothelium-dependent vasodilation, which in turn, causes or exacerbates hypertension.

Oxidation-induced impairment of NO could also result in reduced opposition to the vasoconstrictive and hypertensive effects of angiotensin II.\textsuperscript{16} The expression of eNOS is down-regulated by angiotensin II via protein kinase C, thus leading to a decrease in NO production. In addition, the expression of eNOS may be diminished due to oxidize LDL-C.\textsuperscript{18} Oxidized LDL-C also leads to generation of superoxide anion by endothelial oxidase, which can react with NO yielding a peroxynitrite...
(ONOO−) which is toxic to proteins. In hypercholesterolemia, the levels of asymmetric dimethyl arginine increases which acts as an eNOS endogenous inhibitor.20

The finding of increased levels of MDA in this study has been reported.17,21,22 The increased concentration of MDA levels among hypertensive patients could be due to increased oxidative stress in hypertension. There is greater than normal lipid peroxidation and an imbalance in antioxidant status, suggesting that oxidative stress is important in the pathogenesis of hypertension.22

The significant positive correlation established between MDA and each of age and BMI indicate that oxidative stress progresses with advancing age and body mass index in hypertensive patients from the study area.

Inflammation and oxidative stress act as cooperative and synergistic partners in the pathogenesis of hypertension.22 Inflammation could be due to the primary immune response to eliminate pathogens or to repair tissue damage while innate immune cells, such as neutrophils and macrophages, produce ROS such as superoxide and hydrogen peroxide in order to kill pathogens.23 Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase is a major source of ROS in immune cells and also in the vasculature.23 However, sustained inflammation could lead to an overproduction of ROS.

Oxidative stress is a major cause of endothelial dysfunction, primarily through the reduction of NO bioavailability via the direct chemical reaction of superoxide with NO resulting in the formation of peroxynitrite.24 The reaction between superoxide and NO is faster than the breakdown of superoxide via superoxide dismutase.23 Furthermore, peroxynitrite formation result in further impairment of NO levels and enhanced oxidative stress by inhibiting endothelial nitric oxide synthase (eNOS) activity through oxidation of 4-tetrahydrobiopterin (BH4), a cofactor of eNOS.22

This leads to eNOS uncoupling, where eNOS produces superoxide instead of NO.23 Excessive ROS levels can also induce cellular damage by interacting with DNA, lipids and proteins which may further impair vascular structure and function.22

**CONCLUSION**

Hypertension is a complex disorder that is strongly associated with other risk factors for cardiovascular disease. The aetiology of the association between impaired NO bioactivity, increase MDA and hypertension has not been fully elucidated. Further clarification of the role of impaired NO bioactivity and increased MDA level in hypertension could have important implications for the management of hypertension.

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