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*Full Length Research Paper*

# Oxidative stress and non-enzymic antioxidant status in hypertensive patients in Nigeria

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**Oxidative stress and non-enzymic antioxidant status in plasma of hypertensive patients in Nigeria were investigated. One hundred and fifty hypertensive patients (82 males and 68 females) age range 55 – 75 years visiting Federal Medical Centre, Owerri were selected for the study. Controls were 120 apparently healthy subjects (66 males and 54 females) age range 55 -75 years. Patients with complication such as renal diseases, viral and bacterial infections were excluded from the study. In The hypertensive patients presented significantly higher mean values of plasma total cholesterol, LDL-cholesterol and triacylglycerols and reduced HDL-cholesterol and phospholipids ($p < 0.05$). The result of plasma lipid peroxide was significantly higher in hypertensive patients ($p < 0.05$). Also the levels of non-enzymic antioxidants such as Vitamin C, vitamin E and reduced glutathione in plasma were significantly depleted in the hypertensive patients ($p < 0.05$). This study shows that hypertension is associated with increased oxidative stress and depleted non-enzymic antioxidant status even in developing countries like Nigeria.**

**Key words:** Oxidative stress, non-enzymic antioxidant and hypertension.

## INTRODUCTION

Hypertension is a consistently elevated blood pressure exerted by the blood on the walls of artery. Hypertension is diagnosed when the systolic and diastolic pressure read 140 and 90 mmHg respectively at 3 random checks (Shier, 1999). It is the commonest cardiovascular disease of black Africans (Lawal and Falase, 1988) and a major cause of morbidity and mortality among adult Nigerians (Balogun and Ladipo, 1998). It is one of the leading causes of death and disability due to complications such as coronary heart disease, stroke, congestive heart disease, end-stage renal disease and peripheral vascular disease (Khosh and Khosh, 2001).

Hypertension is correlated with the incidence of artherosclerosis (Barbagallo et al., 1995; Nwanjo, 2005). Previous clinical and epidemiological studies have de-fined plasma lipoprotein levels such as reduced high density lipoprotein-cholesterol (HDL-C), increased total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) etc. as strong predictors of artherosclerosis and hypertension (Austin et al., 1991).

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Investigations have revealed that the concentration of lipid peroxides in plasma correlated well with the severity of atheroselerotic lesion (Stringer et al., 1989). These peroxides are free radicals, which originated from the peroxidations of lipids presumably from plasma membrane peroxylradicals have been incriminated in the pathogenesis of myocardial damage during Ischaemia and the generation of atheroselerotic plagues. In both processes, membrane damage has been considered to play a pivotal role in initiating the cascade process leading to the ultimate cellular death of the affected tissues. Lipid peroxidation is presently been implicated in the pathogenesis of such diseases as diabetes mellitus (Sato et al., 1979) acute myocardial infaction (Loeper et al., 1987), malaria (Hunt and Stocker, 1990), artherosclerosis (steinberg et al., 1989), inflammation (Symons and Dowling, 1987), aging (Pryor, 1982), cancer (Eze et al., 1993) and rheumatoid arthritis (Aruoma, 1993).

The increase in the cellular death of affected tissues not only increases the production of (ROS) but also affects antioxidant reactions catalysed by ROS Scavenging enzymes (Uchimura et al., 1999). All organisms possess antioxidant enzymes (superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GP) responsible

**Table 1.** Changes in plasma lipid profiles in hypertensive patients and control groups.

| Parameter | Control | Hypertensive patients |
|---|---|---|
| Phospholipids (mg/dl) | 5.57± 0.51 | 6.59 ± 0.58* |
| Total Cholesterol (mg/d/) | 147.16 ± 8.18 | 199.89 ± 2.69* |
| HDL-cholesterol (mg/dl) | 51.31 ± 4.55 | 39.49 ± 2.95* |
| LDL-cholesterol (mg/dl) | 98.19 ± 6.83 | 133.26 ± 3.96* |
| Trialcylglycerol (mg/dl) | 89.66 ± 4.81 | 132.98 ± 9.73* |

*Significantly different from control ($p < 0.05$).
Results are means ± S.D.

for scavenging ROS. A defect in ROS scavenging system has been reported in atherosclerosis (Kesavulu et al., 2000). This may also cause decrease in non-enzymic antioxidants.

Thus in the present study, one of the indices of oxidative stress, malondialdalyde (MDA), a bye product of lipid peroxidation and non-enzymic antioxidants were assayed in hypertensive patients with a view to corroborate the findings within our locality (developing country) with findings else where (developing countries). This becomes necessary since manifestation of most diseases vary from locality as exemplified with that of malaria in Indonesia as compared with Southern Sudan (Jensen et al., 1984).

## MATERIALS AND METHODS

One hundred and fifty hypertensive patients (82 males and 68 females) age range 55 – 75 years visiting Federal Medical Centre, Owerri were selected for the study. Controls were 120 apparently healthy subjects (66 males and 54 females) age range 55 - 75 years. Patients with complication such as renal, endocrine or heaptic diseases, diabetes mellitus, obesity, viral and bacterial infections, etc. were excluded from the study. Excluded also were patients taking drugs known to affect lipid metabolism (steroids, diuretics, β-blockers, etc.) were on lipid-lowering therapy within 3 months of starting the study. Hypertension is diagnosed when the systolic and diastolic pressure read 140 and 90 mmHg respectively at 3 random checks (Shier, 1999).

Fresh patient yet to be placed on medication were used since most drugs used for hypertensive treatment are characterized by beneficial lipid changes (Ahaneku et al., 2000).

### Blood sample collection

In all subjects 10 ml blood samples was collected in Na-EDTA (1 mg/ml) tubes after a fasting period of 10 – 12 h. The plasma samples obtained by centrifuging the whole blood in a whisperfuge (model 684) centrifuge at 2500 g for 5 min. In all the samples lipid, lipoprotein and vitamin C assays were performed immediately and samples for other parameters were stored in a freezer below –20°C and were used within 1 month of collection. The lipid peroxide contents of the frozen aliquots did not differ from freshly prepared samples and remained constant throughout the preservation period.

### Estimation of plasma lipids

Total cholesterol, triacylglyceroles as well as HDL cholesterol and phospholipids levels were evaluated using assay kits (purchased from sigma chemical Co., St. Louis, MO, USA). LDL – Cholesterol was calculated using the modified formula of Freidwald (Sandkamp et al., 1990)

### Estimation of plasma lipid peroxidation

Lipid peroxidation in plasma was estimated colorimetrically by measuring malondialdehyde (MDA) by the method of Albro et al. (1986) and Das et al. (1990). In brief, 0.1 ml of plasma was treated with 2 ml of (1:1:1 ratio) TBA–TCA–HCL reagent (TBA 0.37%, 0.25 N HCL and 15% TCA) and placed in water bath for 15 min, cooled and centrifuged and then clear supernatant was measured at 535 nm against reference blank.

### Estimation of non-enzymic antioxidants

Vitamin E (α-tocopherol) was estimated by the method of Desai (1984). In brief, vitamin E was extracted from plasma by addition of 1.6ml ethanol and 2.0ml petroleum ether to 5.0ml plasma and centrifuged. The supernatant was separated and evaporated. To the residue, 0.2ml of 2% α-α-dipyridyl, 0.2ml of 0.5% ferric chloride was added and kept in dark for 5 min. An intense red coloured layer obtained on addition of 4 ml butanol was red at 520 nm.

Vitamin C (ascorbic acid) concentration was measured by Omaye et al. (1979) method. To 0.5 ml of plasma, 1.5 ml of 6% TCA was added and centrifuged (3500g, 2.0min). To 0.5ml of DNPH reagent (2% DNPH) and 4% thiourea in 9 N sulphuric acid was added and incubated for 3 h at room temperature. After incubation 2.5ml of 85% sulphuric acid was added and colour developed was read at 530nm after 30 min.

Reduced glutathione (GSH) was determined by the method of Ellman (1959). 1 ml of supernatant (0.5 ml plasma precipitated by 2 ml of 5% TCA) was taken and 0.5 ml of Ellman's reagent (0.0198% DTNB in 1% sodium citrate) and 3 ml of phosphate buffer (pH 8.0) was added. The colour developed was read at 412 nm.

### Statistics

Statistical evaluation of data was performed by using Duncan's multiple range test (DMRT) (Duncan, 1957).

## RESULTS AND DISCUSSION

Table 1 shows the results of the lipid profile show higher total cholesterol, LDL-cholesterol and triacylglycerole content in hypertensive patients ($p < 0.5$) when compared with the control groups. The HDL-cholesterol and phos-

**Table 2.** Changes in the levels of plasma lipid peroxide, vitamin C, Vitamin E and glutathione in hypertensive patients and control groups.

| Parameters | Control | Hypertensive patients |
|---|---|---|
| Lipid peroxide (mmol/MDA/ml) | 4.11 ± 0.57 | 6.59 ± 0.58* |
| Vitamin C (mg/dl) | 1.29 ± 0.32 | 0.58 ± 0.34* |
| Vitamin E (mg/dl) | 1.62 ± 0.2 | 0.83 ± 0.13* |
| GSH (mg/dl) | 18.64 ± 1.28 | 11.32 ± 1.24* |

*Significantly different from control ($p < 0.05$)
Results are means ± S.D.

pholipids levels were significantly lower in hypertensive patients ($p < 0.5$) when compared with the control.

This study also revealed a significantly higher level of plasma lipid peroxides in hypertensive patients ($p < 0.5$) when compared with control groups (Table 2). The levels of plasma vitamin C, vitamin E and reduced glutathione in hypertensive patients and control are also shown in Table 2. The levels of these non-enzymic antioxidants in plasma were significantly depleted in hypertensive patients ($p < 0.05$) when compared with the control.

Plasma lipid profile, which is altered in hypertensive patients (Loeper, 1987; Steinberg et al., 1989) appears to be a significant factor in the development of premature atherosclerosis and includes an increase in total cholesterol, LDL cholesterol and decrease in HDL cholesterol and phospholipids. Similar result was observed in this study, which shows increase in total cholesterol, LDL cholesterol triacylglycerol and decrease in HDL chole-sterol and phospholipids. It also shows an increase in cholesterol / phospholipid ratio, thereby resulting in disruption of membrane fluidity and leading to membrane alteration of function.

Membrane lipids succumb easily to deleterious actions of reactive oxygen species (Reiler, 1995). The measurement of lipid peroxidation is a convenient method to monitor oxidative damage (Viani et al., 1991). In this study the increase levels of plasma MDA in hypertensive patients reflected the lipid peroxidation as the conesquence of oxidative stress.

Non-enzymic antioxidants such as reduced glutathione, vitamin C and vitamin E play an excellent role in protectting the cells from oxidative damage (Farombi et al., 2000). It is well established that GSH in blood keeps up the cellular levels of the active forms of Vitamin C and Vitamin E by neutralizing the free radicals. When there is reduction in GSH the cellular levels of vitamin C and Vitamin E are closely interlinked to each order. In agreement with this report, the decreased levels of GSH, Vitamin C and Vitamin E in hypertensive patients were observed in this study when compared with the control.

In conclusion, the decrease concentration of non-enzymic antioxidant along with elevated lipid peroxide levels in hypertensive patients could probably be associated with oxidative stress and/or decreased antioxidant defence potential (Mahdi, 2002) even in a developing country like Nigeria.

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