
Ekeanyanwu RC*, Ejiogu RN, Egboagu MC
Department of Biochemistry, Faculty of Science, Imo State University Owerri, Nigeria.

Abstract

A combination of hypertension and diabetes mellitus is known to be accompanied by higher oxidative stress than that observed in the individual disorder alone. The aim of this study is to measure the antioxidant vitamins vitamin C, vitamin E levels and lipid peroxidation status in hypertensive patients and diabetic hypertensive patients receiving vitamin supplements, insulin and lipid lowering drugs. The parameters were correlated with systolic and diastolic blood pressure of the patients. Lipid peroxidation was determined by measurement of Thiobarbituric reactive substance (TBARS) in the serum of patients. The antioxidant status was estimated by determining the levels of vitamin C and vitamin E in serum. A significant (p<0.05) increase in TBARS-MDA level was noticed in the hypertensive patients compared with the normotensive patients. Vitamin E status in hypertensive and diabetic patients were also significantly (p<0.05) lower than that of normotensive patients. However, a significant (p<0.05) decrease in TBARS-MDA levels was observed in the diabetic hypertensive patients compared with the hypertensive patients as well as normotensive patients while the vitamin C level significantly (p<0.05) increased in hypertensive and diabetic hypertensive patients. The present study showed that lipid peroxidation and oxidative stress are altered in hypertension and diabetic hypertension; there are reports that vitamin supplementation and lipid lowering drugs can reverse the effects of these alterations, however, there is no evidence to show that the reactive oxygen species initiated the alterations.

Keywords: Lipid peroxidation; Vitamin C; Vitamin E; TBARS-MDA; Hypertension; Diabetes.

Accepted Dec 29, 2015

Introduction

A global study in the year 2000 showed that 972 million people had hypertension with a prevalence rate of 26.4%. It was projected that this figure will increase to 1.54 billion and a prevalent rate of 29.4% in 2025 [1]. A recent community based study of rural and semi-urban population in Enugu, Nigeria put the prevalence of hypertension in Nigeria at 32.8% [2]. It has been reported that Diabetes and Hypertension coexists in 40-60% of individuals with type 2 diabetes [3,4]. Arauz-Pachec et al. [4,5] reported that diabetic patients have a 1.5-3 times increased prevalence of hypertension compared with non-diabetic patients, with 50% of adults with diabetes reported to have hypertension at time of diagnosis [6]. Recent reports have suggested that hypertension and diabetic complications have a common aetiology involving oxidative stress [7]. A group of authors, Friedman et al. [8] showed that the combination of hypertension and diabetes mellitus is accompanied by higher oxidative stress than that observed in the individual disorder alone.

Oxidative stress occurs when there is an imbalance between the generation of reactive oxygen species (ROS) and the antioxidant defence systems so that the latter become overwhelmed [9]. Hypertension is associated with increased oxidative stress; however, there is still a debate whether oxidative stress is a cause or a result of hypertension. Animal studies have generally supported the hypothesis that, increased blood pressure is associated with increased oxidative stress; however, human studies have been inconsistent [10]. Oxidative stress promotes vascular smooth muscle cell proliferation and hypertrophy and collagen deposition, leading to thickening of the vascular media and narrowing of the vascular lumen. In addition, increased oxidative stress may damage the endothelium-dependent vascular relaxation and increases vascular contractile activity [10].

There is evidence that both free radical production and antioxidant defences are disturbed in diabetes [11]. It has been suggested over the last few years that oxidative stress in diabetes may be partly responsible for the development of diabetic complications [12] and the role of oxidative stress in the pathogenesis of type 1 diabetes mellitus has been implicated in several studies [13-15]. Increased lipid peroxidation products and altered antioxidant enzyme activities were also reported in type 2 diabetes mellitus [16]. Series of clinical and experimental studies have shown that oxidative stress, through free radical generation, plays a role in the onset
Lipid peroxidation and non-enzymatic antioxidants status

of both diabetes [17] and hypertension [18]. Nevertheless, the availability of data is not conclusive and the relationship between hypertension and oxidative stress in diabetic patients remains to be elucidated. This study is aimed to determine the extent of attenuation of oxidative stress in hypertensive and diabetic hypertensive patients receiving vitamin supplements and lipid lowering drugs.

Patients and Methods

Study design and patients

Forty five (45) patients of both sexes and within the age bracket of 49-72 years were included in this study. Previous studies showed that the prevalence of hypertension and diabetes is highest in age groups of 50-69 years [25-27]. The study was conducted on the patients after informed consent was obtained from each of the patients while approval for the study was given by the ethical committee of Federal Medical Centre Owerri, Imo State, Nigeria in accordance with the Helsinki Declaration of 1975 (Edinburgh revision, 2008). Data about the patients for example, age, and so forth was obtained from administered questionnaire and information obtained from their hospital folders and no complaints were encountered during the study. They were subdivided into three main groups as follows:

Group A (Normotensive patients): These were apparently healthy normotensive patients recruited from some of the employees of the Federal Medical Centre Owerri, Imo State. They were 10 in number and served as control patients. They were age and sex matched with the test patients (B and C).

Group B (Hypertensive patients): They were made up of 15 hypertensive patients, reporting to the Clinical Chemistry Department of the Federal Medical Centre Owerri, Imo State for laboratory examination.

Group C (Diabetic Hypertensive Patients): They were made up of 20 hypertensive patients who are diabetics and were reporting to the Clinical Chemistry Department of the Federal Medical Centre Owerri, Imo State, Nigeria for laboratory examination.

Criteria for exclusion

Exclusion criteria were smoking, alcoholics, obesity (body mass index [BMI]>30 kg/m²), hypercholesterolemia and chronic diseases such as kidney failure and cardiovascular disease. It is well known that oxidative stress is elevated in patients with chronic disease such as cardiovascular damage, renal damage. We did not exclude those receiving lipid lowering drugs, insulin and antioxidant vitamins supplements such as mineral ascorbates, probucol, allopurinol quinidine, disopyramide, or other drugs known for affecting serum lipid peroxidation and antioxidant values.

To confirm the hypertensive status of selected patients, blood pressure (BP) was measured using OMRON type 711 Automatic IS Sphygmomanometer. Hypertension was defined as mean daytime blood pressure values ≥ 135mmHg systolic and ≥ 85 mmHg diastolic, by ambulatory blood pressure monitoring [19]. The diagnosis of diabetes was established based on the World Health Organisation diagnostic criteria of the fasting plasma glucose above 126 mg/dl and/or 2-hours post-prandial plasma glucose above 200 mg/dl [20]. The laboratory analysis on collected blood samples was conducted at the Department of Biochemistry Laboratory of Imo State University Owerri, Imo State, Nigeria.

Sample collection

Blood samples were aseptically drawn from an antecubital vein of patients by trained personnel and distributed into each of plain centrifuge tubes for estimation of Serum Glucose, TBARS-MDA, vitamin E and vitamin C. The samples in the plain tubes were allowed to clot. All samples were spun at 1000 rpm for 10 minutes in a Jenlab bench centrifuge model 80-2, and the sera pipetted into serum bottles and analyzed. The serum for serum glucose, TBARS-MDA, vitamin E and vitamin C was separated from packed cells after the samples were spun.

Biochemical assay

Serum Glucose was determined using the modified glucose oxidase method described by Trinder [21]. Vitamin C was determined by the method of Nino and Shah [22], Vitamin E estimation was according to the method described by Fabianek et al. [23] while serum levels of lipid peroxidation were estimated as TBARS and calculated as Malondialdehyde (MDA) according to the method of Okhawa et al. [24].

Data Analysis

Statistical Package for Social Sciences (SPSS), Version 17.0, was used for data analysis. Results were expressed as Mean ± Standard deviation and tests of statistical significance were carried out using one way analysis of variance (ANOVA). Statistical significance was defined as P<0.05. Correlation coefficient between analytes was calculated using Pearson Correlation coefficient at 95% confidence interval.

Results

Table 1 shows the mean values of serum glucose, systolic blood pressure and diastolic blood pressure in normotensive patients; hypertensive patients; diabetic hypertensive patients. The serum glucose level (mg/dl) in the diabetic hypertensive group (214.45 ± 35.58) and the hypertensive group (81.93 ± 15.11) compared to the normotensive patients (82.00 ± 13.32). The systolic blood pressure (mmHg) in the diabetic hypertensive group (170.80 ± 10.76) and the hypertensive group (121.47 ± 5.58) significantly increased (P<0.05) compared to the normotensive patients (115.70 ± 6.91). The diastolic blood pressure (mmHg) in the diabetic hypertensive group (91.35 ± 9.32) and the hypertensive group (75.53 ± 7.92)
showed a significant increase (P<0.05) compared with the normotensive patients.

**Table 1:** Mean levels of serum glucose (SG), systolic blood pressure (SBP), diastolic blood pressure (DBP) in the different groups (A,B, and C).

<table>
<thead>
<tr>
<th>Groups</th>
<th>SG mg/dl</th>
<th>SBP mmHg</th>
<th>DBP mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n=20)</td>
<td>82.00 ± 13.32</td>
<td>115.70 ± 6.91</td>
<td>76.50 ± 5.30</td>
</tr>
<tr>
<td>B (n=30)</td>
<td>81.93 ± 15.11</td>
<td>121.47 ± 5.58</td>
<td>75.53 ± 7.92</td>
</tr>
<tr>
<td>C (n=35)</td>
<td>214.45 ± 35.58</td>
<td>170.80 ± 10.76</td>
<td>91.35 ± 9.32</td>
</tr>
</tbody>
</table>

Table 2 shows the mean values of Thiobarbituric acid reactive substance measured as Malondialdehyde (TBARS-MDA), vitamin C and vitamin E in normotensive patients; hypertensive patients; diabetic hypertensive patients. The diabetic hypertensive group showed significant increase (P<0.05) in the levels of TBARS-MDA (0.16 ± 0.09) and vitamin E (0.49 ± 0.14) compared to the Normotensive groups. The hypertensive group showed significant increase (P<0.05) in the levels of TBARS-MDA (0.28 ± 0.09) and vitamin E (0.87 ± 0.17). However, the levels of TBARS-MDA and vitamin C and vitamin E were significantly decreased (P<0.05) in diabetic hypertensive group compared to the hypertensive group.

**Table 2:** Mean levels of thiobarbituric acid reactive substance (TBARS-MDA), vitamin E, and vitamin C in the different groups (A,B, and C).

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS-MDA mmol/µl</th>
<th>Vitamin C mg/dl</th>
<th>Vitamin E mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n = 20)</td>
<td>0.25 ± 0.08</td>
<td>0.78 ± 0.39</td>
<td>0.92 ± 0.19</td>
</tr>
<tr>
<td>B (n = 30)</td>
<td>0.28 ± 0.29</td>
<td>1.27 ± 0.89</td>
<td>0.87 ± 0.16</td>
</tr>
<tr>
<td>C (n = 35)</td>
<td>0.16 ± 0.09</td>
<td>1.20 ± 0.39</td>
<td>0.49 ± 0.14</td>
</tr>
</tbody>
</table>

Table 3 and Figures 1, 2, 3 depict the relationship between TBARS-MDA, vitamin C and vitamin E and SBP in normotensive patients, hypertensive patients; diabetic hypertensive patients. There is a weak positive correlation (r=0.227) between TBARS-MDA and SBP in diabetic hypertensive patients (Figure 1), but there is a weak negative correlation between vitamin C, vitamin E and SBP in diabetic hypertensive patients (Figure 2 and Figure 3). With regards to hypertensive patients, there is a weak positive correlation (r=0.029) between TBARS-MDA and SBP and a weak negative correlation (r=-0.118, r=-0.335) between vitamin C SBP, vitamin E and SBP.

**Table 4 and figure 4, 5 and 6 depict the relationship between TBARS-MDA, vitamin C and vitamin E and DBP in normotensive patients, hypertensive patients; diabetic hypertensive patients. There is a weak negative correlation (r=-0.206) between TBARS-MDA and DBP in diabetic hypertensive patients (Figure 4). There is also a weak negative correlation (r=-0.244) between vitamin E and DBP in diabetic hypertensive patients (Figure 6). A weak positive correlation (r=0.083) was observed between vitamin C and DBP in diabetic hypertensive patients (Figure 5). With regards to the hypertensive patients, there is a weak positive correlation between TBATS-MDA (r=0.035), vitamin C (r=0.171), vitamin E (r=0.142) and DBP.

**Table 4:** Correlation between DBP and TBARS-MDA, vitamin C, and vitamin E in the different groups (A,B, and C).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBARS-MDA</td>
<td>-0.346</td>
<td>0.328</td>
<td>0.029</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.185</td>
<td>0.608</td>
<td>-0.118</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>-0.144</td>
<td>0.692</td>
<td>-0.335</td>
</tr>
</tbody>
</table>

**Figure 1:** Correlation between TBARS-MDA and SBP in diabetic hypertensive patients. The figure shows that there is a weak positive correlation between systolic blood pressure and TBARS-MDA (r=0.227, P=0.339) in the diabetic hypertensive patients.
Figure 2: Correlation between vitamin C and SPB in diabetic hypertensive patients. The figure shows that there is a weak negative correlation between systolic blood pressure \( (r=-0.041, P=0.861) \) and vitamin C in the diabetic hypertensive patients.

Figure 3: Correlation between vitamin E and SPB in diabetic hypertensive patients. The figure shows that there is a weak negative correlation between systolic blood pressure and vitamin E \( (r=-0.442, P=0.051) \) in the diabetic hypertensive patients.

Figure 4: Correlation between TBARS-MDA and DPB in diabetic hypertensive patients. The figure shows that there is a weak negative correlation between diastolic blood pressure and TBARS-MDA \( (r=-0.206, P=0.384) \) in the diabetic hypertensive patients.

Figure 5: Correlation between vitamin C and DPB in diabetic hypertensive patients. The figure shows that there is a weak positive correlation between diastolic blood pressure and vitamin C \( (r=0.083, P=0.727) \) in the diabetic hypertensive patients.

Figure 6: Correlation between Vitamin E and DPB in diabetic hypertensive patients. The figure shows that there is a weak negative correlation between diastolic blood pressure and vitamin E \( (r=-0.244, P=0.300) \) in the diabetic hypertensive patients.

Discussion and Conclusion

Previous studies showed that the prevalence of hypertension and diabetes is highest in age groups of 50-69 years [25-27]. This formed our decision to limit the study to patients of this particular age bracket. The present findings demonstrate the existence of a relationship between blood pressure and some oxidative stress related parameters. The increased oxidative stress levels observed in hypertensive patients are consistent with the findings of several other studies [28,29] (Simic et al., 2006, Kedziora-kornatowska et al., 2004). Previous findings showed that diabetes and hypertension are associated with increased oxidative stress, which results in higher serum concentration of lipid peroxidation products like TBARS-MDA [25,30,31]. Malondialdehyde is a reliable marker of lipid peroxidation and perioxidative tissue injury [32]. It has been shown to be elevated in animal models of experimentally induced hypertension, suggesting that it is a consequence rather than a cause of hypertension.

In this study, a significant decrease in TBARS-MDA levels was observed in the diabetic hypertensive patients compared with the hypertensive patients and normotensive patients. This is due to the fact that the patients were receiving statin
medication and antidiabetic drugs at the point of reporting to the hospital [33], a confounding factor of oxidative stress [34]. Seghrouchi et al. [35] reported that in patients with type 2 diabetes mellitus, insulin treatment only partially improved oxidative stress parameters. Fava et al. [36] also reported that in type 2 diabetic patients, treatment with glimepiride for 12 weeks ameliorated oxidative stress better than did glibenclamide. However significant increase in TBARS-MDA level was noticed in the hypertensive patients compared with the normotensive patients, suggesting that active lipid peroxidation is occurring in essential hypertension. This is in agreement with various findings by Dhananjay et al. [37]; Nwanjo et al. [38] and Ahmad et al. [39]. Malondialdehyde is a highly toxic by-product, produced in part by oxidation; derived from free radicals. MDA reacts both irreversibly and reversibly with proteins and phospholipids with profound effects and studies have shown significantly raised concentrations in diabetes [40] and hypertension [37,38,39].

The correlation of blood pressure levels with oxidative stress related parameters in normotensive, hypertensive and diabetic hypertensive patients suggests that these parameters have an additional blood pressure modulating effect distinct from those previously observed. Hypertensive and diabetic hypertensive patients showed impairment of the antioxidant defence system as assessed by a diminution of serum antioxidant status, in agreement with previous data [34,41]. Furthermore, the negative correlation between the SBP and both the antioxidant vitamins level points out to the importance of blood antioxidant status in blood pressure modulation.

Non enzymatic antioxidants such as vitamin C and vitamin E play an excellent role in protecting the cells from oxidative damage [42]. Previous findings showed that vitamin C level is lower in hypertensive and diabetic patients compared to general population [25,43,44]. Mayer Davis et al. [45] reported that vitamin C level was unrelated to cardiovascular risk factors in long term while Khaw et al. [46] reported a significant association between plasma vitamin C levels and long term sequels of hypertension. The result of the present finding showed that vitamin C level significantly increased in hypertensive and diabetic hypertensive patients. It has been demonstrated that vitamin C supplementation showed a significant decline in both systolic and diastolic blood pressure which may persist for prolonged period. In addition, vitamin C has been suggested to act more than an antioxidant and its effects on neurotransmitters lead to its antihypertensive activity [47].

Vitamin E status in hypertensive and diabetic patients were significantly lower than that of normotensive patients. Vitamin E is a component of the total peroxyradical-trapping antioxidant system, reacts directly with peroxy and superoxide radicals and singlet oxygen and protects membranes from lipid peroxidation [48]. A study by Bernado Rodriguez-Iturbe et al. [49] demonstrated that an antioxidant-enriched diet that included vitamin E, vitamin C, selenium and zinc reduces the renal interstitial inflammation, decreases renal tissue content of Malondialdehyde and improves hypertension. Furthermore, the cardio-protective potential of vitamin E has been attributed to its potent antioxidant action. This contention is supported by the fact that α-tocopherol shows antioxidant potential by donating hydrogen radical to remove the free radicals reacting with it to form non-radical products or trapping of lipid radicals [50].

In conclusion, from the present study, antioxidant status and lipid peroxidation are altered in hypertension and diabetic hypertension. Although lipid peroxidation decreased in diabetic hypertensive patients receiving vitamin supplements, lipid lowering drugs and insulin, same cannot be said for hypertensive patients. Use of lipid lowering drugs may contribute to the lowering of lipid peroxidation in hypertension. Vitamin E supplementation will produce a beneficial effect in hypertensive and diabetic hypertensive patients. The major limitation of our study was the small sample size. Further studies employing larger population are warranted to further confirm the results of present investigation.

Acknowledgement

Dr. Obinna Chijioke of Federal Medical Centre Owerri is acknowledged for his help in collection of samples. The research study was funded and done by the Undergraduate Research Students of the Department of Biochemistry (2015), Imo State University Owerri who are under the supervision of Dr. Ekeanyanwu, R.C.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

Lipid peroxidation and non-enzymatic antioxidants status


47. Hernandez-Guerra M. Ascorbic acid improves the intrahepatic endothelial dysfunctioning of patients with cirrhosis and portal hypertension. Hepatology, 2006; 43: 485-491.

Correspondence to:
Ekeanyanwu RC
Department of Biochemistry
Faculty of Science
Imo State University
Owerri
Nigeria.