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Idonije O. Blessing
Department of Chemical Pathology, College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria

The Impact of Co-existence of Diabetes and Hypertension on Oxidative Stress in non Obese Subjects

[1]Idonije O. Blessing and [2]Onigbinde O. Abraham

Co-occurrence of diabetes and hypertension is not unfamiliar in the world today and where this co-exists, it is said to aggravate cardiovascular complications. On the other hand, the significance of oxidative stress in cardiovascular condition has been recognized. Hence, this study investigated the impact hypertension and diabetes mellitus has on oxidative stress and the status of some antioxidants in non obese subjects. The study involves 204 non obese subjects who are hypertensive (NOH; n = 53), diabetes (NOD; n = 51), diabetes and hypertensive (NODH; n = 40) and non hypertensive non diabetes (NONDH; control; n = 60). There ages, blood pressure, fasting blood glucose and body mass index were determined using standard techniques while their oxidative stress and antioxidants status were analyzed via standard laboratory procedures. The results showed no significant difference in the ages and BMI in the entire groups. However, blood pressure was significant higher in the hypertensive groups while fasting blood glucose was higher in the diabetes group compared with the control. Oxidative stress was significantly higher ($p<0.05$) in the NOD, NOH and NODH groups compared to the control group (NONDH; 5.32±1.85). Compared to the NONDH group, vitamin A was not significantly different ($p>0.05$) between the groups, vitamin E was significantly higher in group NODH (13.58±5.28) and nitric oxide was significantly lower ($p<0.05$) in the NOH (30.87±3.48) and NODH (30.30±3.13) groups. Judging by the findings of this study, hypertension and diabetes, either existing alone or in combination have a negative impact on oxidative stress and antioxidant status.

Key words: Hypertension, diabetes, co-existence, non obese

[1]Department of Chemical Pathology, College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria
[2]Department of Biochemistry, Faculty of Natural Sciences, Ambrose Alli University, Ekpoma, Edo State, Nigeria

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INTRODUCTION

Diabetic patients have been shown to have increased prevalence of hypertension compared to the general populace (Cowie and Harris, 1995; Anonymous, 1993; Kannel and McGee, 1979). However, patients with co-morbid conditions usually present with one first before the other(s) is/are diagnosed or simultaneously they can co-present and be diagnosed. According to Gress *et al.* (2000), hypertension and diabetes mellitus are independent risk factors for Cardiovascular Diseases (CVD) thereby increasing morbidity and mortality where they co-exist.

It has been reported that hypertension in diabetes accelerates the development and progression of microvascular and macrovascular complications in patients with diabetes (Adler *et al.*, 2000). This is also true in diabetic Nigerians who showed increased mortality with development of hypertension as compared with normotensive diabetic Nigerians (Kolawole and Ajayi, 2000; Ngwogu *et al.*, 2012).

The study of the biochemistry of free radicals has increased since the realization that many major human diseases may involve, in their aetiology, free radical processes and that antioxidant therapy may delay or prevent these processes. Free radicals have been implicated as a contributing factor to the pathophysiology of major chronic diseases such as heart disease and cancer (Halliwell and Gutteridge, 1989; Halliwell *et al.*, 1992). The pathological role of oxidative stress in vascular diseases is well recognized (Madamanchi *et al.*, 2005). Could this be a suggestion that the antioxidants regulatory mechanisms that check oxidative processes are troubled by hypertension and diabetes? Considering the increasing rate of these diseases in the general population and the burden they have on the world economic, it become necessary to investigate what the antioxidants strength and oxidative stress status might be in such conditions as this may provide a clue to their management.

The objective of the study therefore, was to investigate the impact hypertension and diabetes has on oxidative stress and the status of some antioxidants in non obese subjects.

MATERIALS AND METHODS

Study area: This study was conducted in Ekpoma, Benin City, Kwale and Asaba, all in the south-south zone of Nigeria.

Study design: This study is a cross-sectional study involving simple random sampling and cohort sampling for subjects recruitment.

Ethical consideration: Ethical approval was sought and given by the research and Ethic Committee of Ambrose Alli University, Ekpoma. The intervention and control community gave their permission after the aims and objectives of the study were explained to them. Also, informed consent was sought and obtained from the respondents before enrollment into the study. At the end of the study the control group was also given the same intervention, for ethical reasons. Written informed consent/questionnaire were administered to all subjects.

Sampling method and sample size: A simple random sampling was done to recruit subjects for the study. The sample size was determined as described as follows by this formula:

$$N = \frac{Pq}{\left[\frac{\sum}{1.96}\right]^2}$$

Where:

N = Sample size
P = Population or Prevalence rate (50% or 0.5)
q = 1-0.2
Error margin = 0.05

Subjects and grouping: Subjects were classified into the following 4 groups to meet the set goals of this research. Group 1: Non Obese Diabetic (NOD); Group 2: Non Obese Hypertensive (NOH); Group 3: Non Obese Diabetic/Hypertensive (NODH); Group 4: Non Obese Non Diabetic/Hypertensive [normal control(C)].

They were classified as non-obese using BMI$\leq$24.5 kg m^{-2}, diabetic using fasting plasma glucose (FBG)$\geq$7 mmol L^{-1} (126 mg dL^{-1}), hypertensive using blood pressure of $\geq$140/90 mmHg and a total of about two hundred and four subjects were recruited and distributed as follow:

NOD = 51; NOH = 53; NODH = 40; NONDH = 60

Inclusion criteria: All subjects with BMI$\geq$24.5 kg m^{-2} andnot on any form of medication were recruited.

Exclusion criteria: All subjects who are obese (BMI$\geq$24.5 kg m^{-2}) and non obese already diagnosed as been hypertensive and/or diabetic who are on medications were excluded from this study.

Blood pressure measurement: Resting blood pressure was taken in a sitting position after a 5-10 min rest using

a mercury sphygmomanometer according to standard procedures at least four different times and the mean recorded.

BMI measurement: Heights were measured in standing position, with shoulder and buttocks against the wall, the subject looking straight ahead with joined feet and arms hanging on both sides with a graduated tape. In addition, body weights were measured with a calibrated beam scale. These were used to calculate the BMI which is weight (kg)/height (m^2).

Fasting blood glucose (FBG) measurement: Preliminary measurement of FBG in all the subjects was done using Glucometer at least two different times with the mean value recorded. Subjects were asked to fast overnight (no food, drink, alcohol or smoking).

Sample collection and analysis: About 12.5 mL of fasting blood was collected from each subject with 0.5 mL immediately used for preliminary FBG estimation and 2.0 mL immediately placed in fluoride oxalate container for FBG estimation spectrophotometrically as these served as basis for comparing FBG levels. Four (4.0) mL was placed in EDTA container for vitamin A and E and NO, TPP and MDA estimation using standard laboratory procedures.
Vitamin A and E were analyzed using the vitamin A and E high pressure liquid chromatography kits from America laboratory company (Alpco) diagnostics, USA. MDA was analysed using the Spectrophotometry method.

Duration of study: The study was conducted within a thirty months period (from July, 2009 to June, 2012). The first 0-6 months was selection of subjects and baseline measurements of BP and FBG, 7-12 months was repeat measurements of BP and FBG, 13-18 months was repeat measurements of BP and FBG, 19-24 months was final measurements of BP and FBG and collection of sample for analysis and 25-30 months was analysis, collation and processing of data.

Statistical analysis: Data were presented as Mean±SD (standard deviation) and then analyzed using Statistical Package for Social Sciences (SPSS) at a p-value of 0.05 and 95% level of confidence. Where suitable, simple statistics, pair sample t test and the one way analysis of variance were performed intergroup for pair wise comparisons and results presented in suitable tables and charts. Bivariate comparisons were examined using Pearson rank correlation coefficients (r). A $p<0.05$ was considered significant.

RESULTS

Table 1 is the Mean±standard deviation of ages, blood pressure, fasting blood glucose levels and body mass index of the different groups in the sampled population. The NOH group showed a mean age of 51.34±11.88 years and was the oldest while those in NONDH group were the youngest with a mean age of 42.33±14.64 years. The entire groups were within the normal ranges of BMI (kg m^{-2}) with the NOH group (23.51±1.69 kg m^{-2}) presenting the lowest and the NODH group (24.15±2.27 kg m^{-2}) presenting the highest. Statistically, there was no significant different in the ages and BMI of the sampled population between the groups. On blood pressure, the systolic and diastolic blood pressure of the NOH and NODH groups were higher (168.20±17.74/98.13±9.81 and 163.50±21.44/ 97.73±11.43 mmHg, respectively) and these were significant ($p<0.05$) compared to the NONDH group (125.50±10.14/70.77±11.29 mmHg) and the NOD group (132.50±9.81/78.47±10.99 mmHg). On the other hand, fasting blood glucose was highest in the NODH group (11.32±3.30), higher in the NOD group (11.04±3.30) and high in the NOH group (4.83±1.30) compared to the NONDH group (3.93±0.85). These differences in FBG were statistically significant ($p<0.05$) in the NOD, NOH and NODH groups compared with the control.

Table 2 shows the level of oxidative stress in the studied population indicated via MDA status and antioxidant level. Compared to the control group (NONDH; 5.32±1.85), the NOD, NOH and NODH groups showed significantly higher ($p<0.05$) MDA levels. On the antioxidant status, the level of vitamin A was not

Table 1: Mean age, blood pressure, FBG and BMI in Mean±SD of the sampled population

Parameter	NOD (n=51)	NOH (n=53)	NODH (n=40)	NONDH (n=60)
AGE (years)	45.69±13.96	51.34±11.88	48.45±10.50	42.33±14.64
BMI (kg m^{-2})	23.75±2.700	23.51±1.690	24.15±2.270	23.62±2.030
BP systolic (mmHg)	132.50±9.810	168.20±17.74*	163.5±21.44*	125.50±10.14
BP diastolic (mmHg)	78.47±10.99	98.13±9.81*	97.73±11.43*	70.77±11.29
FBG (xxx)	11.04±3.30*	4.83±1.30*	11.32±3.30*	3.93±0.850

*Significantly different at $p<0.05$ from the control (NONDH)

Table 2: Biomarkers of oxidative stress and antioxidant status in Mean±SD of non obese diabetes and hypertensive subjects compared with control (apparently healthy; NONDH)

Parameter	NOD	NOH	NODH	NONDH
MDA	6.49±1.51*	6.28±1.72*	6.86±1.79*	5.32±1.85
Vit. A	0.45±0.22	0.47±0.26	0.42±0.21	0.45±0.23
Vit. E	13.41±5.28*	11.86±5.47	13.58±5.28*	11.66±4.27
NO	31.04±2.82	30.87±3.48*	30.30±3.13*	32.37±2.53

*Significantly different at $p<0.05$ from the control (NONDH)

significantly different ($p>0.05$) between the groups. However, vitamin E was significantly higher in NODH group (13.58±5.28) compared to the NONDH group (11.66±4.27) and others. In addition, the value of nitric oxide was significantly lower ($p<0.05$) in the NOH (30.87±3.48) and NODH (30.30±3.13) groups compared to the NONDH (32.37±2.53) and the NOD (31.04±2.82) groups.

DISCUSSION

The present study indicated that diabetes and hypertension potentiate oxidative stress and where they co-exist, oxidative stress is severely aggravated. In addition, according to the present results, diabetes has more potential in worsening oxidative stress compared to hypertension. In accordance with the present finding, increase in oxidative stress has been reported in human hypertension (Kashyap *et al*., 2005; Sun *et al*., 2006; Lip *et al*., 2003) and diabetes (Maddux *et al*., 2001; Rudich *et al*., 1998; Matsuoka *et al*., 1997) and these were based on increased levels of biomarkers of lipid peroxidation and oxidative stress.

Although, studies have reported oxidative stress to play critical roles in the pathogenesis of many diseases (Brownlee, 2001), the suggestion that it impair insulin secretion by pancreatic β cells (Matsuoka *et al*., 1997) and glucose transport in muscle (Maddux *et al*., 2001) may explained the finding in the NOD and NODH groups. Extended exposure to high levels of Reactive Oxygen Species (ROS) can damage DNA, proteins and lipids (Bate, 2004). Hence, long term diabetes and hypertensive exposure may be more detrimental to health. In line with this assertion, increased oxidative stress in vascular walls has been shown to be involved in the pathogenesis of hypertension (Nakazono *et al*., 1991), atherosclerosis (Ohara *et al*., 1993) and hepatic steatosis (Roskams *et al*., 2003) and this may explained the contribution by the NOH and NODH groups. Putting together these information by Matsuoka *et al*. (1997), Maddux *et al*. (2001), Nakazono *et al*. (1991), Ohara *et al*. (1993) and Roskams *et al*. (2003), one cannot but support the aggravated MDA level in co-existence of diabetes and hypertension as showed by the NODH group. Indeed, it has been previously shown by studies that the co-existence of the two conditions is a powerful promoter of CVD, accelerating microvascular and macrovascular complications and greatly increasing cardiovascular stroke and end stage renal disease risk (Sowers, 2004; Sampanis and Zamboulis, 2008).

In terms of antioxidant status, the present study showed that the level of vitamin A was not significantly influence by diabetes and or hypertension, the level of vitamin E and nitric oxide were affected by the presence of diabetes and hypertension and this effect was severe where they co-exist. This finding is in line with previous reports (Kashyap *et al*., 2005; Sun *et al*., 2006) that vascular diseases lower antioxidants capacity judging by the significant reduction in nitric oxide level which is recognized as a potent inhibitor of the lipid peroxidation. In agreement with this, hypertensive condition has been reported to lower Total Antioxidant Capacity (TAC) levels (Kashyap *et al*., 2005; Sun *et al*., 2006).

In accordance with the finding of this study on the influence of diabetes and hypertension on MDA, decreased antioxidant activity and reduced levels of Reactive Oxygen Species (ROS) scavengers (vitamins E and C and glutathione) have been reported to contribute to oxidative stress (Taddei *et al*., 1998). Although studies have revealed no clear benefit of antioxidant supplementation no improvement in blood pressure (Heart Protection Collaborative Group 2002; Vievkananthan *et al*., 2003), the present study however indicated that hypertension and diabetes influence vitamin E and nitric oxide levels. Also, short-term oral high-doses of zinc, vitamin C, β-carotene and alpha-tocopherol have been reported by Galley *et al*. (1997) to lower blood pressure, possibly via increased availability of nitric oxide. Furthermore glucose; which is a component of diabetes, has been reported to alter the balance between free radicals and nitric oxide in endothelial cells by nitric oxide exerting its vasodilatory and antioxidant effect and this deleterious effect is said to occurs in endothelial cells exposed to glucose, which, in fact, favours the formation of oxygen and in turn promotes oxidation (Cosentino *et al*., 1997). Hence, the reduce nitric oxide which acts as a potent inhibitor of the lipid peroxidation chain reaction by scavenging propagatory lipid peroxyl radicals, reported in this study may not be unrelated to the presence of hypertension and diabetes.

The increase oxidative stress status and reduction in nitric oxide reported in this study by the hypertensive and diabetes conditions either existing alone or in combination may be the contributory factors to cardiovascular diseases. This fact is supported when one view the pathological role of oxidative stress in vascular diseases as previously stated by Madamanchi *et al*. (2005).

CONCLUSION

Conclusively, judging by the findings of this study, hypertension and diabetes, either existing alone or in

combination have a negative impact on oxidative stress and antioxidant status. By implication, the reported CVD reported among diabetes and hypertension may be related to the impact these conditions have on oxidative stress and antioxidant status.

REFERENCES

Adler, A.I., I.M. Stratton, H.A.W. Neil, J.S. Yudkin and D.R. Matthews *et al*., 2000. Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes rospective observational study. Br. Med. J., 321: 412-419.

Anonymous, 1993. Hypertension in Diabetes Study (HDS): I. Prevalence of hypertension in newly presenting type 2 diabetic patients and the association with risk factors for cardiovascular and diabetic complications. J. Hypertens., 11: 309-317.

Bate, A.N.O., 2004. Stage dependent response of malaria parasite anti-oxidant defense enzymes and heat shock proteins on oxidatively-stressed parasites. University of Turin

Brownlee, M., 2001. Biochemistry and molecular cell biology of diabetic complications. Nature, 414: 813-820.

Cosentino, F., K. Hishikawa, Z.S. Katusic and T.F. Luscher, 1997. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation, 96: 25-28.

Cowie, C.C. and M.I. Harris, 1995. Physical and Metabolic Characteristics of Persons with Diabetes. In: Diabetes in America, Harris, M.I., C.C. Cowie, M.P. Stern, E.J. Boyko, G.E. Reiber and P.H. Bennett (Eds.). 2nd Edn. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Washington, DC., USA.

Galley, H.F., J. Thornton, P.D. Howdle, B.E. Walker and N.R. Webster, 1997. Combination oral antioxidant supplementation reduces blood pressure. Clin. Sci., 92: 361-365.

Gress, T.W., F.J. Nieto, E. Shahar, M.R. Wofford and F.L. Brancati, 2000. Hypertension and antihypertensive therapy as risk factors for type 2 diabetes mellitus. N. Engl. J. Med., 342: 905-912.

Halliwell, B. and J.M.C. Gutteridge, 1989. Free Radicals in Biology and Medicine. 2nd Edn., Clarendon Press, Oxford.

Halliwell, B., J.M. Gutteridge and C.E. Cross, 1992. Free radicals, antioxidants and human disease: Where are we now?. J. Lab. Clin. Med., 119: 598-620.

Heart Protection Collaborative Group, 2002. MRC/BHF heart protection study of antioxidant vitamin supplementation in 20,536 high-risk individuals: A randomised placebo-controlled trial. Lancet, 360: 23-33.

Kannel, W.B. and D.L. McGee, 1979. Diabetes and cardiovascular disease. The Framingham study. JAMA, 241: 2035-2038.

Kashyap, M.K., V. Yadav, B.S. Sherawat, S. Jain and S. Kumari *et al*., 2005. Different antioxidants status, total antioxidant power and free radicals in essential hypertension. Mol. Cell Biochem., 277: 89-99.

Kolawole, B.A. and A.A.L. Ajayi, 2000. Prognostic indices for intra-hospital mortality in Nigerian diabetic NIDDM patients: Role of gender and hypertension. J. Diabetes Complications, 14: 84-89.

Lip, G.Y., M.J. Laudary, E. Edmunds and B.A. Hughes, 2003. Abnormal LDL Subtractions profile in patients with untreated hypertension. Qs Med., 95: 165-171.

Madamanchi, N.R., A. Vendrov and M.S. Runge, 2005. Oxidative stress and vascular diseases. Arteriosecler. Thromb. Vasc. Biol., 25: 29-38.

Maddux, B.A., W. See, J.C. Lawrence, Jr., A.L. Goldfine, I.D. Goldfine and J.L. Evans, 2001. Protection against oxidative stress-induced insulin resistance in rat L6 muscle cells by micromolar concentrations of α-lipoic acid. Diabetes, 50: 404-410.

Matsuoka, T., Y. Kajimoto, H. Watada, H. Kaneto and M. Kishimoto *et al*., 1997. Glycation-dependent, reactive oxygen species-mediated suppression of the insulin gene promoter activity in HIT cells. J. Clin. Invest., 99: 144-150.

Nakazono, K., N. Watanabe, K. Matsuno, J. Sasaki, T. Sato and M. Inoue, 1991. Does superoxide underlie the pathogenesis of hypertension? Proc. Natl. Acad. Sci. USA., 88: 10045-10048.

Ngwogu, K.O., B.O. Ekpo, F.C. Akpuaka, A.C. Ngwogu and O. Okhiai, 2012. The prevalence of obesity as indicated by body mass index among apparently healthy adults living in Aba, Abia State, Nigeria. Int. J. Basic Applied Innov. Res., 1: 19-25.

Ohara, Y., T.E. Peterson and D.G. Harrison, 1993. Hypercholesterolemia increases endothelial superoxide anion production. J. Clin. Invest., 91: 2546-2551.

Roskams, T., S.Q. Yang, A. Koteish, A. Durnez and R. DeVos *et al*., 2003. Oxidative stress and oval cell accumulation in mice and humans with alcoholic and nonalcoholic fatty liver disease. Am. J. Pathol., 163: 1301-1311.

Rudich, A., A. Tirosh, R. Potashnik, R. Hemi, H. Kanety and N. Bashan, 1998. Prolonged oxidative stress impairs insulin-induced GLUT4 translocation in 3T3-L1 adipocytes. Diabetes, 47: 1562-1569.

Sampanis, C. and C. Zamboulis, 2008. Arterial hypertension in diabetes mellitus: From theory to clinical practice. Hippokratia, 12: 74-80.

Sowers, J.R., 2004. Insulin resistance and hypertension. Am. J. Physiol. Heart Circ. Physiol., 286: H1597-H1602.

Sun, L., Y.H. Gao, D.K. Tian, J.P. Zheng, C.Y. Zhu, Y. Ke and K. Bian, 2006. Inflammation of different tissues in spontaneously hypertensive rats. Acta Physiologica Sinica, 58: 318-323.

Taddei, S., A. Virdis, L. Ghiadoni, A. Magagna and A. Salvetti, 1998. Vitamin C improves endothelium-dependent vasodilation by restoring nitric oxide activity in essential hypertension. Circulation, 97: 2222-2229.

Vievkananthan, D.P., M.S. Penn, S.K. Sapp, A. Hsu and E.J. Topol, 2003. Use of antioxidant vitamins for the prevention of cardiovascular disease: Met-analysis of randomized trials. Lancet, 361: 2017-2023.