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Metabolic Syndrome and Oxidative Stress Among Patients of Type 2 Diabetes in an African Community

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Abstract: *Background*: This study was conducted to determine the prevalence of metabolic syndrome and oxidative stress among type 2 diabetes mellitus (DM) patients in Enugu metropolis. *Materials and Methods*: Two hundred and forty participants aged 40-80 years attending Diabetic Clinic, University of Nigeria Teaching Hospital, Nigeria with their age and gender-marched healthy individuals were recruited as subjects and controls respectively, and evaluated for metabolic syndrome using the National Cholesterol Education Programme-Adult Treatment Panel III criteria. Oxidative stress markers were assayed spectrophotometrically. *Results*: Prevalence of metabolic syndrome was found to be 56% among type 2 DM patients (55% for males and 58% for females), and 15% among apparently healthy individuals. Besides raised fasting blood glucose in the subjects, the second abnormal parameter was raised blood pressure (86%), followed by low high-density lipoproteins (45%), central obesity (33%), and raised fasting triglycerides (17%) in that particular order. Furthermore, oxidative stress markers were found to have changed significantly in subjects when compared to controls: Malondialdehyde values and superoxide dismutase activities were significantly higher while ascorbic acid values were significantly lower. *Conclusion*: It can be concluded from this study that metabolic syndrome and disturbances of reduction-oxidation homeostasis are common among type 2 DM patients.

Keywords: Metabolic Syndrome, Oxidative Stress, Type 2 Diabetes

1. Introduction

Metabolic syndrome is a constellation of some medical disorders, which has type 2 diabetes mellitus (DM) as one of its risk factors. It is a cluster comprising hyperglycaemia, dyslipidaemia, hypertension, and central obesity [1, 2]. The syndrome is a medical condition with many hypotheses in attempting to explain its pathophysiology. However the most acceptable and unifying of them all is the insulin resistance hypothesis, which states that an incompletely understood defect in insulin action is the underlying pathology in the condition [1, 3] The insulin resistance is preceded by postprandial hyperinsulinaemia, followed by hyperinsulinaemia and eventually hyperglycaemia [3]. Consequently, there is a postulation of a strong association between metabolic syndrome and type 2 DM. Oxidative stress, on the other hand, refers to a shift towards the pro-oxidant/antioxidant imbalance that can occur as a result of an increase in oxidative metabolism. In oxidative stress, there is generation of highly reactive unstable molecules and atoms that deplete the antioxidant level of the body. So simply put oxidative stress results when pro-oxidants exceed antioxidants in the body [4].

Type 2 DM is characterized by impaired insulin secretion, insulin resistance, excessive hepatic glucose production, and

deranged fat metabolism. In the early stages of the disease, glucose tolerance remains near-normal despite insulin resistance due to the compensatory role of pancreatic beta cells leading to increased insulin output. With worsening insulin resistance and progression of compensatory hyperinsulinaemia, the pancreatic islets in certain people are unable to sustain the hyperinsulinaemia, which results to impaired glucose tolerance, a state of elevated postprandial glucose. A further decline in insulin secretion and an increase in hepatic glucose production lead to overt diabetes with fasting hyperglycaemia and eventually, beta cells failure arises [5]. Although insulin resistance and abnormal insulin secretion are central to the development of type 2DM, the disease likely encompasses a range of disorders with common the phenotype of hyperglycaemia.

Most of our current understanding of the pathophysiology and genetics is based on studies of individuals of European descent. It is becoming increasingly clear that DM in other ethnic groups (Africans, Asians, and Latin Americans) has a different but yet to be identified pathophysiology [6]. Consequently, this research work is to determine the prevalence of metabolic syndrome and the presence of oxidative stress among black patients with type 2 DM and to compare the data with that of the western populations.

2. Materials and Methods

2.1. Study Design/Location/Area

The research was a case-controlled cross-sectional study that involved the determination of some biophysical and biochemical parameters among controls and subjects for diagnosis of metabolic syndrome using the NCEP-ATP III criteria and comparison of markers of oxidative stress between subjects and controls.

The study was carried out at the Diabetic Clinic of University of Nigeria Teaching Hospital (UNTH) Enugu and controls are residents of Enugu metropolis. All laboratory analyses were done in the Department of Chemical Pathology of the hospital.

2.2. Ethical Consideration

Ethical clearance was obtained from the Institutional Human Research Ethics Committee with approval reference NHREC/05/01/2008B before the commencement of the study, oral and written informed consent was obtained from each participant before enrolment into the study.

2.3. Study Population

The study subjects consisted of men and women aged 40-80 years of age diagnosed with type 2 DM of less than five years duration and who are attendees of the Diabetic Clinic Unit, Medical Out-patient Department of UNTH, Enugu state, Nigeria.

2.4. Sample Size Determination/Sampling Technique

The sample size was determined using the formula described by Araoye [7]. The sampling technique was by the convenient sampling technique where participants who consented and satisfied the inclusion criteria were recruited.

2.5. Biophysical Measurements

Blood pressure and waist circumference measurement of subjects and control were carried out using the indirect sphygmomanometry and use of tape method respectively [8]. The indirect sphygmomanometric method of blood pressure measurement involved the use of appropriate cuff over the right arm, inflated over the brachial artery, allowed to deflate, and readings at first and fifth Korokoff's sounds was noted as systolic and diastolic blood pressures, respectively [9].

Use of tape for waist circumference measurement involved measuring abdominal girth to the nearest 0.5cm on the bare skin of a standing participant around points just above the iliac crests.

2.6. Collection and Processing of Blood Samples

Fasting blood samples were collected with 5 ml syringes after the cubital has been disinfected and tied with the tourniquet. About 2 ml of blood was collected into fluoride bottles and the remaining 3 ml dispensed into sterile plain tubes. The samples in plain tubes were allowed to clot for 20 minutes and spun at 5,000 rpm for 5 minutes. The sera (supernatant) was separated and analyzed for triglycerides, HDL-cholesterol, malondialdehyde, vitamin C, superoxide dismutase. Some sera not analyzed immediately were stored frozen at 0°C. On the other hand, plasma from fluoride bottles was used for the analysis of fasting blood glucose concentration.

2.7. Determination of Biochemical Parameters

Blood glucose concentration was determined using the enzymatic method as outlined by Trinder [10]. Triacylglycerol concentration was determined using the enzymatic method outlined by Buccolo and David [11] while the precipitation method as outlined by Burstein *et al* [12] was used for HDL-cholesterol concentration determination. Superoxide dismutase activity was assayed by the enzymatic assay technique as outlined by McCord and Fridovich [13].

Vitamin C concentration was determined according to the method of Pearson [14]. Lipid peroxidation was determined by measuring spectrophotometrically the concentration of malondialdehyde (MDA) as described by Wallin *et al* [15].

2.8. Definition of Metabolic Syndrome

The criteria for diagnosing metabolic syndrome have evolved since its original definition in 1999. For this research, the National Cholesterol Education Programme-Adult Treatment Panel III (NCEP-ATP III) 2012 was used, which states that a diagnosis of metabolic syndrome is made when three or more of the following conditions are present in an individual: central obesity (waist circumference > 102 cm for males or > 88 cm for females), hypertriglyceridemia (serum triglycerides ≥ 150 mg/dl), low high-density lipoprotein-cholesterol (HDL-cholesterol < 40 mg/dl for males or 50 mg/dl for females), hypertension (systolic blood

pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 85 mmHg) or hyperglycaemia (fasting blood glucose ≥ 100 mg/dl [2].

2.9. Statistical Analyses

The data obtained were analyzed using Statistical Product and Service Solutions (SPSS), version 23. Continuous data were initially evaluated for conformity to normal distribution using the Shapiro-Wilks tests. Those found not to be of normal distribution were log-transformed before analysis. The results, following analysis, were expressed as mean±standard deviation and the significant differences of the results were established by T-test values or one-way analysis of variance (ANOVA), when necessary. The categorical data were expressed in counts/percentages. The prevalence of metabolic syndrome, central obesity, hypertension, hyperglycaemia, hypertriglyceridaemia, and low HDL-cholesterol were calculated as follows: prevalence (%) = number of subjects with condition/total number of subjects. The acceptance level of significance for the results was $p < 0.05$.

3. Results

Table 1. *Biophysical and Biochemical Characteristics of Participants.*

PARAMETER	CONTROLS	CASE SUBJECTS
Number (n)	120	120
Age (years)	62.7±24.8	67.4±19.5
Waist Circumference (cm)	86.58±9.86*	90.11±10.35*
Blood pressure (BP), mmHg		
Systolic BP	131.08±12.11*	150.65±15.25*
Diastolic BP	77.03±6.01*	86.58±9.86*
Fasting blood glucose (mg/dl)	89.91±12.95*	123.77±30.02*
HDL-cholesterol (mg/dl)	52.33±8.26*	45.73±8.79*
Triglycerides (mg/dl)	139.04±9.19*	143.45±11.68*
Malodialdehyde (mg/dl)	6.98±0.73*	7.95±0.52*
Ascorbic acid (mg/dl)	1.58±0.14*	1.23±0.26*
Superoxide dismutase (IU/ml)	60.48±5.58*	94.02±19.90*

*Statistically significant ($p < 0.05$).
BP: Blood pressure
HDL: High-density lipoprotein.

Table 2. *Prevalence of some conditions among the Participants.*

CONDITION n (%)	CONTROLS n (%)	CASE SUBJECTS n (%)
Central obesity	21 (18)	40 (33)
Hypertension	55 (46)	103 (86)
Hyperglycaemia	27 (23)	120 (100)
Low HDL –cholesterol	-	54 (45)
Hypertriglyceridaemia	-	21 (17)
Metabolic syndrome	18 (15)	67 (56)

HDL: High-density lipoprotein.

Table 3. *Prevalence of some conditions between male and female participants.*

CONDITIONS	CONTROLS		CASE SUBJECTS	
	Male (n=60) n (%)	Female (n=60) n (%)	Male (n=60) (%)	Female (n=60) n (%)
Central obesity	9 (15)	12 (20)	10 (17)	30 (50)
Hypertension	28 (47)	27 (45)	49 (82)	45 (75)
Hyperglycaemia	15 (25)	12 (20)	60 (100)	60 (100)
Low HDL-cholesterol	-	-	31 (52)	23 (38)
Hypertriglyceridaemia	-	-	8 (13)	12 (20)
Metabolic syndrome	10 (17)	7 (12)	33 (55)	35 (58)

HDL: High-density lipoprotein.

Table 1 shows the biophysical and biochemical parameters of the participants. 120 each of controls and subjects was recruited with age ranges of 62.7±24.8 and 67.4±19.5 (in years) respectively. All the measured parameters: waist circumference, blood pressure, fasting blood glucose, HDL-cholesterol, triglycerides, malondialdehyde, and superoxide dismutase enzyme showed a significantly increased levels among the subjects when compared to the controls whereas ascorbic acid showed a significantly decreased level.

Table 2 shows the prevalence of some conditions among the participants. It showed that all the subjects had hyperglycaemia with a prevalence of 100%, followed by hypertension (86%), low HDL-cholesterol (54%), central obesity (33%), and hypertriglyceridaemia (17%). Among the control subjects, hypertension was more prevalent (46%)

followed by hyperglycaemia (23%) and central obesity (18) whereas none of them had dyslipidaemia (low HDL-C or high TG). The prevalence of metabolic syndrome was 67% for the subjects and 15% for the controls.

Table 3 shows the prevalence of these conditions among male and female participants. For all the other conditions, the males (whether subjects or controls) had higher prevalence rates except for the central obesity status. More women were centrally obsessed (with 50% prevalence among the subjects and 20% prevalence for controls).

4. Discussion

Among the type 2 DM subjects, the prevalence of metabolic syndrome was 56% (55% for males and 58% for females). These findings differed from results from similar study in Nnewi within the same Southeast region of Nigeria, where they found the prevalence of metabolic syndrome in type 2 DM patients to be 66.7% (41.9% for males and 58.1% for females) [16]. This slight difference in prevalence rate may be attributed to differences in research designs: in this study only type 2 DM patients of 5 years or less duration was recruited but in the cited research such consideration was not made.

Among the healthy individuals who served as controls, the prevalence of metabolic syndrome was found to be 15%, which was very similar to finding in a study in Ibadan in which the prevalence of apparently healthy individuals was found to be 16.8% [17]. This is worrisome because it was an incidental finding in which the individuals did not know they had any medical condition; this underscores the need for regular medical check-ups.

Of the five parameters measured, aside hyperglycaemia, which had a prevalence of 100% for the subjects, the second predominant abnormal parameter was raised blood pressure with a prevalence of 86%. This finding was in agreement with several other studies [18, 19]. Low HDL-cholesterol accounted for the third abnormal parameter with a prevalence of 45% (more for men 52% and women 38%). Central obesity showed a prevalence of 33% (more in women 50% and men 17%). Hypertriglyceridaemia was the least abnormal finding with a prevalence of 17%. This order of abnormalities was similar to findings in some other studies [19, 20]. However, from an earlier study conducted in Nnewi, it was observed that low HDL-cholesterol and hypertriglyceridaemia were third and fourth while central obesity was the fifth abnormal parameter [16].

For the oxidative stress markers, malondialdehyde (MDA) and ascorbic acid were measured, and also the activity of superoxide dismutase (SOD) enzyme was assayed. The MDA values of test subjects were significantly higher when compared to the control subjects. Also there were significantly higher values of MDA for both sexes as their ages increased. This is in agreement with the findings from a similar study [21].

Moreover, ascorbic acid values were significantly lower for test subjects when compared to control subjects. Also, there were progressively significantly lowered ascorbic acid values as the subjects became older in both sexes, which showed a strong negative correlation. The results were in agreement with the findings from another similar study [22].

These findings could be explained by the fact that as an antioxidant, it would be depleted in conditions of increased oxidative stress as found in metabolic syndrome.

Furthermore, superoxide dismutase activity was found to be significantly higher in test subjects as compared to control subjects. It was also significantly higher as their ages increased. These findings are in agreement with a similar study cited earlier [21], where it was argued that it could be as a result of compensatory mechanisms to counter the oxidative stress effect of reactive oxygen species generated. However, the finding differed from that of two other studies [5, 23] in which superoxide dismutase activity was significantly lower. The different findings may be explained in that they assayed SOD contents of the ultrafiltrates from cells (intracellular value) as against that of blood (extracellular; intravascular value). In oxidative stress, there is associated cellular injury and death leading to the release of intracellular constituents into the vascular space.

In any case, the study has shown that in type 2 DM and metabolic syndrome there is depletion of antioxidants and increased lipid peroxidation. The changes have also been shown to be worse as they get older but not related to their sex differences.

5. Conclusion

Metabolic syndrome was found to be highly prevalent (about 56%) among type 2 DM patients and to some extent common (about 15%) in apparently healthy individuals that served as controls. There was also a strong association of the syndrome with aging. There was however no significant difference in the prevalence of the different sexes of the test subjects. But female subjects tend to have more of central obesity than male subjects, while the male subjects had more of dyslipidaemia (particularly low HDL-cholesterol) than female subjects.

Additionally, oxidative stress markers were found to have changed significantly (MDA increased, ascorbic acid decreased while SOD increased).

Given the above findings, the followings are recommended:

1. Increased research work to see variations of known biochemical parameters in our localities.
2. The concept of metabolic syndrome has gained prominence because it aims at detecting and monitoring incipient sufferers at very early stages of diabetes and hypertension. So, regular hospital visits are to be encouraged in our communities.

Disclosure Statement

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Authors' Contributions

All authors were involved substantially in the concept and design of the study, data acquisition, analysis and interpretation of the data, drafting the article, revising the article critically for its intellectual content, and in the final approval of the version to be published.

Data Availability

The data analyzed and used in this study may be shared with other researchers on reasonable request provided the data comply with the same standards as the main dataset.

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