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Evaluation of male sex hormones and oxidative stress markers in obese subjects with and without metabolic syndrome in Nnewi Nigeria

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ABSTRACT

Background

The incidence of metabolic syndrome is rapidly increasing in Nigeria and it is a cluster of risk factors of abdominal obesity, dyslipidaemia, hyperglycaemia, hypertension which can lead to infertility and oxidative stress. The objective of this study was to verify the influence of metabolic syndrome on male fertility hormones, oxidative stress and antioxidant defense in obese subjects.

Materials and Method

This is a cross sectional study in which hundred (100) obese subjects between the ages of 29 – 49 yrs were recruited. They were divided into two groups; Group 1 obese with metabolic syndrome 50 subjects, and Group 2 obese without metabolic syndrome 50 subjects. The classification of metabolic syndrome was based on the National Cholesterol Education Programe ATP III guidelines. Fasting blood samples were collected for biochemical analyses. Serum metabolic profile and oxidative stress markers were analysed colorimetrically while serum level of male sex hormones and insulin were measured with Enzyme immunosorbent assay method. Statistical analyses was done using SPSS version 23.0.

Result

The mean level of metabolic profile (fasting plasma glucose, fasting blood insulin homeostatic model assessment – Insulin Resistance index, total cholesterol, Low density lipoproteins, Very Low Density Lipoprotein and triglyceride) were significantly higher (P<0.05) in groups with metabolic syndrome. Furthermore, there were significant lower level of testosterone and higher level of estradiol in metabolic syndrome group (P<0.05). The mean levels of antioxidants total antioxidant capacity and Glutathione Peroxidase were not significantly lower in test group (metabolic syndrome) when compared with the control (P>0.05), however, malondialdehyde was significantly higher (P<0.05).

Conclusion

In conclusion, components of metabolic syndrome can cause alteration in male sex hormones thus leading to infertility Also it can cause a redox imbalance characterized by increased plasma oxidation and reduced antioxidant capacity.

Keywords: Metabolic Syndrome, Fertility Hormones, Obesity, Insulin Resistance, Oxidative Stress

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INTRODUCTION

There is a gradual rise in obesity and high prevalence of metabolic syndrome (MetS) in obese subjects in Nigerian young adults.[1] Obesity plays a pivotal role in metabolic syndrome (MetS), a constellation of clinico-biochemical parameters that places an individual at increased risk for cardiovascular disease. The syndrome is characterized by the presence of any three or more of obesity, hyperglycaemia, hypertension, and atherogenic dyslipidaemia (hypertriglyceridaemia), many other metabolic diseases like male or female infertility.[2,3] Metabolic syndromes has been associated with insulin resistance.[4] Close links exist between metabolic syndrome and oxidative stress due to an imbalance between pro-oxidant and anti-oxidant species in favour of oxidised entities. Insulin resistance and inflammation increase reactive oxygen species (ROS) production and oxidative stress through superoxide anion production and the decreased levels of antioxidative enzymes such as superoxide dismutase and glutathione peroxidase.[5,6] This leads to end products of lipid peroxidation such as malondialdehyde (MDA).[6] Although oxidative stress is considered the underlying mechanism by which dysfunctional metabolism occurs in obese subjects, there are few studies on evaluation of oxidative stress in obese subjects with metabolic syndrome in our environment. In the setting of an increasing prevalence and understanding of metabolic syndrome, researchers are actively studying the potential relationship between metabolic syndrome and male factor infertility.[7,8] Many studies have demonstrated the condition of imbalance in the hypothalamic- and pituitary-gonadal axis in obese male with the outcome of significant depression in total testosterone and sex hormone binding globulin. Supplementary acquisition particularly addressing the components of metabolic syndrome and their impact on male reproduction will enhance our understanding of the underlying pathophysiology. There has been paucity and conflicting findings on association of infertility and oxidative stress with metabolic syndrome in our environment so this research aims at evaluating oxidative stress markers and male sex hormones in metabolic obese subjects.

MATERIAL AND METHODS

Study Site/Design

The research was carried out at Nnewi, Anambra state, Nigeria and biochemical analyses were performed at Nnamdi Azikiwe Teaching Hospital (NAUTH) Nnewi, Anambra State, Eastern Nigeria. This hospital was chosen because they have competent personnel (Medical laboratory scientist) and equipment.

Hundred obese subjects were randomly recruited and were further examined to identify subjects with metabolic syndrome according to the National Cholesterol Education Program Adult Treatment Panel III NCEP (2001) definition.

Clinical Identification of Metabolic Syndrome

According to the National Cholesterol Education Program Adult Treatment Panel III NCEP (2001)[9] definitions, metabolic syndrome is present if three or more of the following five criteria are met:

1) Central obesity: waist circumference ≥ 102 cm or 40 inches (male)
2) Dyslipidemia: TG ≥ 1.7 mmol/L (150 mg/dl)
3) Dyslipidemia: HDL-C < 1.03 mmol/L (40 mg/dL) (male),
4) Blood pressure ≥ 130/85 mmHg
5) Fasting plasma glucose ≥ 6.1 mmol/L (110 mg/dl)

Inclusion and Exclusion Criteria

Subjects recruited were between the ages of 29 and 47years with body mass index of 30 - 40Kg/m^2. Apparently healthy individuals who were not on any medications for diabetes, hypertension and other CVD were recruited. Subjects on alcohol, cigarette, children, adolescents, normal weight and overweight subject, morbid obese (BMI above 41Kg/m^2), bedridden, physically challenged, and subjects above 50 years were excluded from the study.

Ethical Approval and Informed Consent

Ethical approval was sought and obtained from the Research Ethics Committee of the Nnamdi Azikiwe University Teaching hospital (RECNAUTH) Nnewi, Anambra state with reference NAUTH/CS/66/VOL10/2017/010. The participants were informed about the study designs and their

written informed consent was obtained before they were recruited.

Data Collection Procedure

Subjects who indicated interest in the study, following discussion at business areas, churches, offices, recreation outlets, and restaurant were given detailed designed questionnaire to fill.

Anthropometric Measurement

The weights of the subjects were evaluated with scale (Gulfex Medical and Scientific, England)). The subjects' heights were recorded in meters using a height scale calibrated in centimeters. As a measure of generalized obesity, each adult participant's BMI was computed by dividing the weight in kilograms, by the square of the height in meters (kg/m^2). To determine abdominal obesity, measurement of the waist circumference (WC) was taken using a stretch-resistant tape (HTS, China). Blood pressure (BP) systolic and diastolic pressure readings were taken from the participant's left arm using sphygmomanometer (Omron Medical, United Kingdom). The reading was taken in the morning to the nearest mmHg.

Sample Collection, Storage and Analysis

5mls of blood sample was collected from fasting subjects between 8 and 10am using standard procedure as described by Lewis et al., (2006).[10] 1ml of whole blood was dispensed into fluoride oxalate bottle and the plasma separated for glucose analysis while the remaining 4ml of whole blood was dispensed into plain bottle and allowed to clot, retracted and spun at 3000RPM for 10minutes after which the serum was separated into two aliquots and stored. Plasma glucose was analyzed as soon as possible while serum if not assayed immediately was stored at -20°C not more than 2weeks before analyses.

Glucose was assayed colorimetrically using Glucose oxidase method of Trinder, (1969).[11] Lipid profile, malondealdehyde (MDA), Total Antioxidant Capacity (TAC) and Gluthathione peroxidise (GPx) level was determined by colorimetric method. The LH, FSH, Prolactin, Estradiol, testosterone, serum insulin level were estimated based on solid phase enzyme linked immunosorbent assay (ELISA) method using ACUBIND kit and mindray (MR- 96A) ELISA machine. Insulin Resistance (IR), was assessed by homoeostasis model assessment–insulin-resistance index (HOMA–IR), according to the following formulas: 'fasting insulin value (mU/L) × fasting blood sugar level (mmol/L) / 22.5' , values exceeding 2.25 would denote insulin resistance.[12] Quality control was ensured by using pooled control sera from apparently healthy individual and commercially purchased control (Randox (USA) Control level.

Statistical Analyses

Statistical analyses were performed using statistical package for social sciences (SPSS) software version 23.0 software. The independent student t- test was used to assess significant mean difference between two variables metabolic and non metabolic syndrome group and level of significant was considered at $P<0.05$.

RESULTS

Considering the components of metabolic syndrome, abdominal obesity 47 (94%), was the most prevalent in obese metabolic syndrome individual studied, hypertension 30(60%), hyperglycaemia 16(32%), being the least, hypertriglyceridaemia 19(38%), Low HDL-C was 17 (34%).

Anthropometric and Blood Pressure Level in Metabolic and Non-Metabolic Syndrome Subject

Although age, weight, height and BMI were higher in obese with metabolic syndrome (MS+) they were not significant ($P>0.05$) whereas waist circumference, systolic and diastolic syndrome were significantly higher in the test group ($P<0.05$) as shown in Table 1.

Fasting Plasma Glucose, Fasting Blood Insulin, HOMA-IR and Lipid Profile Level in Metabolic and Non-Metabolic Syndrome Group

The mean level of fasting blood glucose, fasting blood insulin and HOMA- IR, total cholesterol, LDL-C, VLDL- C and triglyceride were significantly higher ($P<0.05$) in groups with metabolic syndrome when compared with those without the syndrome as shown in Table 2.

Table 1 Anthropometric and Blood Pressure Measurement in Metabolic and Non-Metabolic Syndrome Group Subject

Parameters	Metabolic Syndrome (N=50)	No Metabolic Syndrome (N=50)	T-Test	P-Value
AGE (yrs)	38.9±5.6	38.4±5.4	0 .48	0.634
WEIGHT (kg)	104±10.9	102.6±10	0 .58	0.563
HEIGHT (m)	1.70±.07	1.71±.07	-0.25	0.802
BMI	35.75±3.0	35.20±2.7	0.86	0.393
WAIST CIRCUMFERENCE (cm)	113±7.6	108±6.5	3.20	0.002*
SBP (mmHg)	138.9±14.7	127±9.3	4.40	0.000*
DBP (mmHg)	92.9±9.5	86.7±7.6	3.30	0.002*

**=Significant at P<0.05, SBP=Systolic Blood Pressure, DBP=Diastolic Blood Pressure, BMI= Body Mass Index*

Table 2 Fasting Plasma Glucose, Fasting Blood Insulin, HOMA-IR and Lipid Profile Level in in Metabolic and Non-Metabolic Syndrome Group

Parameters	Metabolic Syndrome (N=50)	No Metabolic Syndrome (N=50)	T-Test	P-Value
FBG (mmol/L)	6.0±.84	5.4±.67	3.9	0.000*
FBI (µIu/ml)	8.8±1.3	6.2±3.7	7.1	0.000*
HOMA-IR	2.3±.50	1.5±.44	8.1	0.000*
TC (mmol/L)	6.5±1.3	5.9±.83	2.5	0.014*
LDL-C (mmol/L)	4.4±1.5	3.8±1.0	2.2	0.032*
HDL-C (mmol/L)	1.2±.43	1.3±.34	-1.9	0.065
VLDL-C (mmol/L)	.85±.22	.64±.15	5.1	0.000*
TRIG (mmol/L)	1.9±.48	1.4±.32	4.9	0.000*

**=Significant at P<0.05, FBG= Fasting Blood Glucose, HOMA-IR Homeostatic Model Assessment-Insulin Resistance, TCHOL-C, Total cholesterol, LDL-C= Low Density Lipoprotein cholesterol, HDL-C= High Density Cholesterol, VLDL-C= Very Low Density Cholesterol, TRIG= Triglyceride.*

Fertility Hormones, Malondialdehyde, Total Antioxidant Capacity and Glutathione Peroxidase Level in Metabolic and Non-Metabolic Syndrome Group

Estradiol level was significantly higher and testosterone lower in metabolic syndrome group when compared with (MS-) (P<0.05). Furthermore, though luteinizing hormones and Follicular Stimulating hormones levels were lower and prolactin higher in test group, they were not significant.

The mean levels of antioxidant TAC and GPX though lower in test group (metabolic syndrome) when compared with the control were not significant (P>0.05), however MDA was significantly higher in MetS group (P<0.05) as shown in Table 3.

In Table 4, insulin resistance shows significant positive correlation with, prolactin, estradiol, and significant negative correlation with testosterone, LH, FSH and HDL (P<0.05). The waist circumference shows significant positive correlation with, estradiol, and significant negative correlation with testosterone, LH and FSH (P<0.05). No significant positive correlation was seen in Prolactin (P>0.05).

Table 3 Fertility Hormones, Oxidative Stress Markers Levels in Metabolic and Non-Metabolic Syndrome Subjects

PARAMETER	Metabolic Syndrome (N=50)	No-Metabolic Syndrome (N=50)	T-test	P-value
LH (mIU/ml)	5.3±1.6	5.1±1.7	.593	0.555
FSH (mIU/ml)	6.4±2.1	5.8±2.1	1.22	0.225
PROLACTIN (ng/ml)	8.2±2.0	8.0±2.3	.331	0.742
TESTOSTERONE(ng/ml)	5.8±2.2	7.1±2.6	-2.3	0.024*
ESTRADIOL (pg/ml)	107±35.3	91±15	2.83	0.006*
MDA (nmol/L)	4.0±1.0	3.5±1.2	2.10	0.037*
GPX (U/ml)	1.03±.21	1.08±.18	-1.09	0.277
TAC (µmol/L)	943±149	961±196	-0.468	0.641

**=Significant at P<0.05, LH= Luteinizing Hormone, FSH= Follicular Stimulating Hormone, TAC = Total Antioxidant Capacity*

Table 4 Correlation of HOMA-IR and Waist Circumference with Fertility Hormones in Male Obese Individuals

Parameter	HOMA-IR		Waist Circumference	
	r	P-Value	r	P-Value
LH (mIU/ml)	-0.177	0.036*	-0.241	0.004*
FSH (mIU/ml)	-0.178	0.035*	-0.270	0.001*
PROLACTIN (ng/ml)	.190	0.025*	0.165	0.052
TESTOTERONE(ng/ml)	-0.210	0.013*	-0.337	0.000*
ESTRADIOL (pg/ml)	0.347	0.001*	0.477	0.000*

LH= Leutinizing Hormones, FSH= Follicular Stimulating Hormones

In Table 5, insulin resistance shows significant positive correlation with waist circumference SBP, DBP, FBG, insulin (P<0.05). However, no significant negative correlation was observed with GPx and TAC (P>0.05). MDA though showed positive correlation was not significant.

The waist circumference shows significant positive correlation with SBP, DBP, FBG, MDA, insulin, and significant negative correlation with TAC, (P<0.05).

Table 5 Correlation of HOMA-IR and Waist Circumference with Blood Pressure, FPG, Insulin and Oxidative Stress Markers in Male Obese Individuals

Parameter	HOMA-IR		Waist Circumference	
	r	P-Value	r	P-Value
WAIST (cm)	0.566	0.000*	1.000	0.000*
SBP (mm/Hg)	0.491	0.000*	0.459	0.000*
DBP (mm/Hg)	0.325	0.001*	0.506	0.000*
FBG (mmol/L)	0.379	0.000*	0.362	0.000*
MDA (nmol/ml)	0.115	0.117	0.221	0.009*
TAC (µmol/L)	-0.145	0.087	-0.212	0.012*
GPX (U/ml)	-0.083	0.331	-0.140	0.098
Insulin (uIU/ml)	0.928	0.000	0.335	0.004*

**=Significant at P<0.05 SBP= Systolic Blood Pressure, DBP= Diastolic Blood Pressure, FPG= Fasting Plasma Glucose, MDA= Malondealdehyde, TAC= Total Antioxidant Capacity*

DISCUSSION

Metabolic Syndrome has become a public health problem throughout the world and is defined by a combination of several metabolic abnormalities.[4] In this study, abdominal obesity was the most prevalence 47 (94%) among all the components of metabolic syndrome examined followed by hypertension 29 (58%). Abdominal obesity is significantly associated with metabolic syndrome, and this was observed in this study where there was significant increase in waist circumference in MetS obese individual when compared with MS-. Also on correlating waist circumference with some of the components of metabolic syndrome, there was strong association. This observation shows that abdominal obesity is associated with hypertension, hyperglycemia, oxidative stress and infertility.

Plasma glucose, HOMA_IR, insulin level, systolic and diastolic blood pressure also significantly were higher in MetS obese subject when compared with those without confirming it being clusters of risk factor of hyperglycemia, hypertension and insulin resistance ($P<0.05$).

Insulin resistance and hyperinsulinemia are the primary underlying metabolic abnormalities reported in metabolic syndrome which was also observed in this study after its correlation with other components of MetS. 12 obese were insulin resistance (HOMA-IR≥ 2.65) of which 10 were from (MetS) group and 2 from (MS-). Observational studies report that low levels of testosterone and SHBG are significantly correlated with MetS and its associated components (including measures of BMI, waist circumference, and waist-height ratio).[13] Growing evidence has linked metabolic syndrome and its individual symptoms to the increasing prevalence of male infertility.[14] Though this study showed that LH, FSH, were not significantly lower and prolactin higher in MetS individual when compared with MS- ($P>0.05$), testosterone level was lower and estradiol level higher in metabolic syndrome group ($P<0.05$). In the aforementioned studies, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were normal or low in obese men, they argue that even normal levels of gonadotropins in the context of low free testosterone signify suppression of the hypothalamic-pituitary axis, resulting in subclinical hypogonadotropic hypogonadism.[15,16] Thus, the observed decrease in testosterone levels in obese metabolic males is likely due to several factors, including decreased synthesis of testosterone, inhibition of SHBG synthesis, and decreased gonadotropin secretion.[17, 18] This is in line with work done by some researchers who also observed the inverse correlation between metabolic syndrome with both sex hormone-binding globulin and testosterone.[7, 13]

Numerous researchers have noted that, the components of metabolic syndrome are associated with systemic proinflammatory states and increased oxidative stress with lipid peroxidation.[19] This present study showed that oxidative stress is increased and antioxidant defense is decreased in obese metabolic syndrome subjects due to increased lipid peroxidation. This is in line with work done by Venturini et al.[19] which showed that overweight subjects with MetS, in contrast to overweight subjects without MetS, have a redox imbalance characterized by increased plasma oxidation and reduced antioxidant capacity. Subjects suffering from MetS seem to have higher inflammation status (CRP) and a higher level of oxidative stress, which is characterized by the increase in MDA levels.[20-22] A state of chronic low-level inflammation and oxidative stress demonstrates a close link to MetS.[23] Taken together, these data suggest that – although weight gain or visceral fat may contribute, to some extent, to an increase in oxidative stress – the presence of MetS is fundamental in showing reactive oxygen species augmentation in overweight and obese subjects leading to infertility.[24]

CONCLUSION

In conclusion, metabolic syndrome is associated with infertility due to male sex alteration and oxidative stress caused by the components of the metabolic syndrome.

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