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## Oxidative Stress Biomarkers and Their Relationship with Testosterone in Male Auto Mechanics in Ibadan, Nigeria

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## Authors' contributions

This work was carried out in collaboration between all authors. All authors designed the study, participated in the writing of the protocol, read and approved the final manuscript. Authors SAA and SUO managed the recruitment of the participants and performed the biophysical measurements, biochemical and statistical analyses. Author SAA wrote the first draft. Authors SAA, MACD and AAO managed the literature, interpreted the data and critically reviewed the manuscript.

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## ABSTRACT

Occupational exposure to mixed chemicals generates free radicals with inadequate antioxidants resulting in oxidative stress. Recently, hypogonadism in male auto-mechanics was associated with oxidative stress. Studies show that testosterone, a male hormone increases the activities of antioxidant enzymes. This study is aimed at evaluating the oxidative stress biomarkers and their relationship with testosterone in auto mechanics in Ibadan, Nigeria.

Eighty-three males participated in this prospective cross sectional study after informed consent. Forty-three were male auto-mechanics, occupationally exposed to mixed chemicals in the mechanic community, Bodija, Ibadan (cases). Their mean (SEM) age and body mass index (BMI) were 42.5 (1.7) years and 23.8 (0.5) Kg/m<sup>2</sup> respectively. They were age and BMI matched with 40 unexposed, apparently healthy males from the University College Hospital and environs (controls). Demography, social habits, anthropometry and gonadal status were obtained by standard methods. Serum obtained from blood (10 ml) collected from the participants was used for biochemical

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analyses. Testosterone levels were determined by enzyme immunoassay method (Immunometrics UK Ltd). Levels of total antioxidant capacity, total plasma peroxide (TPP), malondialdehyde (MDA), hydrogen peroxide ( $H_2O_2$ ), glutathione peroxidase (GPX), superoxide dismutase (SOD), glutathione-S-transferase (GST), and reduced glutathione (GSH) were determined using spectrophotometric methods while oxidative stress index (OSI) was calculated. *P*<0.05 was regarded as significant.

TPP, MDA, OSI,  $H_2O_2$  and GST `levels were significantly higher (*P*<0.001) in eugonadal cases compared with controls. All these biomarkers levels were similar in hypogonadal compared with eugonadal cases. (*P*>0.05) Testosterone related negatively with SOD in the controls only but positively with MDA and negatively with GST in cases only (*P*<0.05).

Occupationally exposed auto mechanics appear to have oxidative stress and may benefit improvement in antioxidant status. Testosterone may contribute to and enhance total antioxidant status, which may be important in gonadal function.

Keywords: Mixed chemical; oxidative stress; antioxidants; hypogonadism; occupational exposure.

## 1. INTRODUCTION

Infertility affects 8-15% of couples in their reproductive age worldwide with more prevalence in Central and Southern African countries known as the infertility belt [1,2]. Male factor infertility is an emerging health problem worldwide with prevalence of 40% in Nigeria [3]. Idiopathic infertility is the most common cause of male infertility and oxidative stress may be vital in its aetiology [2]. Oxidative stress is an imbalance between free radical generation and antioxidant defence system [4]. It is increasingly being recognized as a possible mechanism in the toxicity of various chemicals exposure at the work place including heavy metals (lead, cadmium, arsenic, mercury), organic and inorganic solvents (including chloroform, alcohol, toluene, alkalis, ether, petrol), gases (including ammonia, chlorine, hydrogen sulphide) and acids (sulphuric acid, hydrochloric acid) [5,6].

Total plasma peroxide (TPP) is the sum of all hydrogen peroxides and other derivatives of peroxides produced physiologically in the body, occurring in higher concentration in some pathological conditions [7]. Quantification of lipid peroxidation is essential to assess the role of oxidative injury in pathophysiological disorders [8-10]. Oxidative stress index (OSI) is a tool to assess the oxidative (redox) status of an individual and markers of initiation and progression of numerous diseases. OSI is a combined measurement depicted by the ratio between pro-oxidants and antioxidants [11].

Individual oxidative biomarkers may be important in assessing subtle alteration in antioxidant defence system. Hydrogen peroxide  $(H_2O_2)$ reacts with reduced transition metals such as iron, via the Fenton reaction, to produce the highly reactive hydroxyl radical [12]. Malondialdehyde (MDA) is a three carbon, low molecular weight aldehyde that can be produced from free radical attack on polysaturated fatty acids of biological membranes, and used for monitoring lipid peroxidation in biological samples [13].

Antioxidants have been known to retard lipid oxidation through competitive binding of oxygen, retardation of the initiation step, blocking the propagation step by destroying or binding free radicals, inhibition of catalysts, or stabilization of hydroperoxides [14]. Total antioxidant capacity (TAC) is described as the sum of all known endogenous and exogenous antioxidants in a medium [15]. It is commonly used to estimate oxidative stress levels in a system [16]. Its reduction may simply elevate reactive oxygen species (ROS) implying exhausted antioxidant defence, which is unable to scavenge ROS and neutralize their toxic effects [17]. Increased TAC has been associated with successful fertilization, suggesting that a decreased TAC may play a pathogenetic role in male infertility [18,19].

Exposure to mixed chemicals increases lipid peroxidation due to free radical generation and causes antioxidant enzymes depletion, and alterations in antioxidant defence system. These include enzyme activities such as glutathione peroxidase (GPX), glutathione-S-transferase (GST), superoxide dismutase (SOD) and nonenzymatic molecule like glutathione, thereby, resulting in oxidative cellular damage in the testes [20,21].

SOD is an endogenously produced intracellular enzyme that is fundamental in the process of eliminating ROS by reducing superoxide to form hydrogen peroxide [22]. GPX reduces hydrogen peroxide to water by oxidizing glutathione (GSH) [10]. GSH is an antioxidant that prevents damage to important cellular components caused by ROS such as free radicals and peroxides [23]. GST catalyses the conjugation of glutathione and acts as an antioxidant by detoxifing peroxidised lipids [24].

Sex hormones have antioxidant effects, which decrease oxidant production in different cells. and this effect may be of importance in the protection against free radical mediated diseases [25,26]. Testosterone is an androgenic and anabolic hormone that is primarily secreted in the testes, ovaries, adrenal glands and skin. It is important in the modulation of every component involved in erectile function, regulates sexual behaviour and enhances reproduction [27,28]. Hypogonadism is abnormally low testosterone production, which may occur because of testicular dysfunction (primary hypogonadism) or hypothalamic-pituitary dysfunction (secondary hypogonadism), and may be congenital or acquired [29].

Adequate antioxidants may be necessary for adequate gonadal function in males occupationally exposed to mixed chemicals. Testosterone has been shown to have direct antioxidant effects by increasing the activities of antioxidant enzymes such as glutathione peroxidase, hence contributing to antioxidant capacity [30]. Reduction in TAC significantly correlated with total testosterone in males [31]. Okoli et al. [32] recently reported significant hypogonadism associated with reduced TAC, implicating oxidative stress in auto-mechanics occupational exposed to mixed chemicals. Their findings were however, confounded by increased abdominal obesity, a known cause of hypogonadism [33,34] in the occupationally exposed non-obese males.

In Nigeria, the contribution of specific oxidative stress biomarkers to hypogonadism in occupationally exposed auto mechanics is uncertain. The aim of this study is to evaluate oxidative stress biomarkers and their relationship with testosterone in male auto mechanics exposed to mixed chemicals in a mechanic community in Ibadan.

## 2. MATERIALS AND METHODS

#### 2.1 Study Design

This is a prospective cross-sectional study conducted in auto mechanics in the mechanic

community in Bodija, Ibadan. Ethical approval was obtained from UI/UCH Ethical Committee.

#### 2.2 Study Population

A total of 83 males participated in this prospective cross sectional study after informed consent. 43 were male auto-mechanics, occupationally exposed to mixed chemicals in the mechanic community, Bodija, Ibadan with mean (SEM) age and body mass index (BMI) of 42.5 (1.7) years and 23.8 (0.5) Kg/m<sup>2</sup> respectively (cases). They were age {38.7 (1.3) years; P=0.067} and BMI matched {24.1 (2.8) Kg/m<sup>2;</sup> P=0.654} with <u>40</u> apparently healthy males from the University College Hospital and environs, living and working outside the mechanic village and unexposed to mixed chemicals (controls). Those with on medications, multivitamins, food supplements and any known chronic illnesses that increase oxidative stress such as diabetes mellitus, cardiovascular disease or any form of neurodegenerative disease were excluded from the study.

#### 2.3 Sample Collection

10 ml of venous blood sample were aseptically collected by venepuncture from participants. This was done by applying a tourniquet 4-6 inches (10-15 cm) above the intended puncture site to obstruct the return of venous blood to the heart and to distend the vein. The site of the puncture, the medial cubital vein in the antecubital fossa was cleansed with alcohol swab. Blood was then collected with new disposable pyrogen free needles and syringes after the skin has air dried. was dispensed into plain serum tubes and kept for 1-2 hours to clot. The blood samples were centrifuged at 500 g for five minutes after which serum was obtained and stored in small aliquots at -20℃ until analyses were done. Serum obtained was used for hormonal indices and oxidative stress biomarkers.

#### 2.4 Demography and Social Habits

Demographic indices (educational status, marital status and parity), social habits (smoking history, alcohol consumption) and duration of occupational exposure to mixed chemicals (DOEMC) were obtained from semi structured pre-test questionnaire administered to the study participants.

#### 2.5 Anthropometry

Waist circumference (WC), hip circumference (HC) and waist hip ratio (WHR) were measured

and calculated as described elsewhere [33,35]. The values obtained in this study in cases versus controls were 86.7 (1.7) cm, 93.6 (1.4) cm, 0.9 (0.1) versus 82.1(1.0), 95.1 (1.3), 0.8 (0.1) respectively already reported by Okoli et al. [32].

#### 2.6 Gonadal Status of EMC and Controls

Gonadal status of the participants was done as described by Emokpe et al. [3]. All the controls were eugonadic (100%), However, thirty (69%), 6 (13.4%), 4 (9.3%) and 3 (6.9%) cases had eugonadism, hypogonadotropic hypogonadism (HH), compensatory hypogonadism and suboptimal hypogonadism respectively. Testosterone levels in cases versus controls were 13.0 (0.6) nmol/L versus 12.0 (0.9) respectively [32].

#### 2.7 Oxidative Stress Biomarkers

Oxidative stress biomarkers estimated were TAC, TPP, MDA,  $H_2O_2$ , SOD, GPX, GST and GSH while OSI was calculated. Measurement of TAC was carried out by using the ferric reducing antioxidant power (FRAP) assay of Benzie and Strain [36]. TAC levels in cases versus controls were 1032 (70) µm/L versus 1186 (44.6) µm/L respectively [32]. TPP levels were determined using the ferrous oxidation (FOX2) method [37] with minor modifications [38,39]. OSI was calculated as shown below [40].

$$OSI (in \%) = \frac{TPP (\mu mol H2O2)}{TAC (\mu mol/L)} \times 100$$

GPX was estimated using enzymatic method by Rotruck et al. [41]. Hydrogen peroxide was estimated spectrophotometrically using Wolff's method [42]. SOD activity was determined by the method of Misra and Fridovich [43]. GST activity was determined according to Habig et al. [44]. GSH was measured by the method of Beutler et al. [45]. Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation [46].

### 2.8 Statistical Analysis

Statistical Package for Social Science (SPSS) 17.0 was used to analyze the data. Student's ttest was used to test the significant differences between mean values. Multiple regression analysis was employed to find relationships between the quantitative variables while Chisquare test was used to find associations. Data obtained were significant at P<0.05.

## 3. RESULTS

# 3.1 Oxidative Stress Biomarkers in Cases and Controls

Table 1 compares the mean (SEM) oxidative stress biomarkers between cases and controls. TPP, OSI, MDA,  $H_2O_2$  and GST were significantly higher in the cases compared with controls (*P*<0.001).

#### 3.2 Oxidative Stress Biomarkers in Eugonadic Cases and Controls

Table 2 compares the mean (SEM) oxidative stress biomarkers between eugonadic cases and controls. TPP, OSI, MDA,  $H_2O_2$ , GST were significantly higher in eugonadic cases compared with controls (*P*<.05).

## 3.3 Oxidative Stress Biomarkers in Eugonadic and Hypogonadotrophic Hypogonadic Cases

Table 3 shows comparison of mean (SEM) oxidative stress biomarkers between eugonadic and hypogonadotropic cases. All the oxidative stress biomarkers were similar in both groups (P>.05).

### 3.4 Demography, Social Habits and Occupational Exposure to Mixed Chemicals in Cases and Controls

Table 4 compares the demographic and social habits of cases with controls. The cases appear less educated than the controls (P<.001). The mean (SEM) of DOEMC was 21.2 (1.9) years while the duration at work was 10.5 (0.3) hours. No significant association was observed in cigarette smoking and alcohol between cases and controls (P>0.05).

## 3.5 Relationship of Testosterone with Oxidative Biomarkers and Duration of Occupational Exposure with Anthropometry in Cases and Controls

Table 5 shows regression of testosterone with LH (32) and oxidative biomarkers (including TAC) in cases and controls. The regression was not a good fit ( $R^2$  adjusted = 8.6%) and the overall relationship was not significant (*P*=.226) in the cases. However, testosterone was

positively related with MDA ( $\beta$ = 0.13, *P*=.043) and negatively related to GST ( $\beta$ = -2.45, *P*=.049). Similarly, the regression of testosterone with LH and oxidative biomarkers in controls was

not a good fit ( $R^2$  adjusted = 10.1%) and the overall relationship was not significant (P= .05). However, testosterone was negatively related with SOD ( $\beta$ = -7.52, P=.02).

Table 1. Comparison of oxidative stress biomarkers between participants exposed to mixed chemicals and unexposed to mixed chemicals

Oxidative stress biomarkers	Cases n=43	Controls n=40	P
TPP (µmolH <sub>2</sub> 0 <sub>2</sub> /L)	9.7 (0.4)	4.8 (0.1)	<.001*
OSI (%)	1.2 (0.1)	0.4 (0.0)	<.001*
MDA(µmol/L)	30.9 (1.7)	17.9 (0.9)	<.001*
$H_2O_2$ (µmoles)	10.8 (0.4)	4.1 (0.3)	<.001*
GPX (U/ml)	8.9 (0.1)	9.1 (0.1)	.252
SOD (U/ml)	1.5 (0.1)	1.6 (0.1)	.238
GST (µmol/ml)	1.2 (0.1)	0.8 (0.04)	<.001*
GSH (Ug/ml)	18.5 (1.3)	16.7 (1.2)	0.326

Values are in mean (SEM), TPP=total plasma peroxide, p=probability, OSI=oxidative stress index, GPX=glutathione peroxidase, \*= significant, MDA=malondialdehyde, SOD=superoxide dismutase, H2O2=hydrogen peroxide, GST=glutathione-S-transferase, GSH=reduced glutathione

Table 2. Com	parison of	oxidative	stress	indices	between	eugonadic	cases an	d controls
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Oxidative stress biomarkers	s Eugonadic c <mark>ases</mark>	Controls	Р
	n = 30	n = 40	
TPP(µmolH <sub>2</sub> 0 <sub>2</sub> /L)	10.1 (2.4)	4.7 (0.9)	.000*
OSI (%)	1.2 (0.6)	0.4 (0.1)	<.001*
MDA (µmol/L)	31.9 (1.2)	17.9 (5.8)	<.001*
$H_2O_2$ (µmoles)	10 <mark>.6 (3.</mark> 1)	4.1 (1.8)	<.001*
GPX (U/ml)	8.9 (0.9)	9.1 (0.8)	.204
SOD (U/ml)	1.4 (0.5)	1.6 (0.3)	.203
GST (µmol/ml)	1.2 (0.4)	0.8 (0.3)	<.001*
GSH (Ug/ml)	17.3 (7.0)	16.7 (7.8)	.736

Values are in mean (SEM), TPP = Total plasma peroxide, P= probability, \*= statistically significant, OSI = Oxidative stress index, GPX = Glutathione Peroxidase, MDA = malondialdehyde, SOD = superoxide dismutase, H<sub>2</sub>O<sub>2</sub> = Hydrogen peroxide, GST=glutathione-S-transferase, GSH=reduced glutathione

Table 3. C	Dxidative	stress bi	iomarkers	between	eugonadic	and hypo	gonadotrophi	С
			hypog	onadic c	ases			

Oxidative stress biomarkers	Eugonadic cases n = 30	Hypogonadotrophic hypogonadic cases n = 6	Р
TPP (µmolH <sub>2</sub> 0 <sub>2</sub> /L)	10.1 (2.4)	7.9 (1.7)	.050
OSI (%)	1.2 (0.6)	1.0 (0.5)	.454
MDA (µmol/L)	31.9 (11.2)	30.0 (12.8)	.722
$H_2O_2$ (µmoles)	10.6 (3.1)	10.8 (2.6)	.866
GPX (U/ml)	8.9 (0.9)	8.6 (0.8)	.438
SOD (U/ml)	1.4 (0.5)	1.4 (0.6)	.689
GST (µmol/ml)	1.2 (0.4)	1.4 (0.6)	.304
GSH (Ug/ml)	17.3 (7.0)	22.1 (10.7)	.172

Values are in mean (SEM), TPP = total plasma peroxide, P= probability \*= statistically significant, OSI = oxidative stress index, GPX = glutathione peroxidase, MDA = malondialdehyde, SOD = superoxide dismutase, H<sub>2</sub>O<sub>2</sub> = hydrogen peroxide, GST=glutathione-S-transferase, GSH=reduced glutathione

	Cases	Controls	X²	Р
	n=43	n=40		
Demography				
Marital status	Single = 6 (14%)	Single = 2 (5%)		
	Married = 37 (86%)	Married = 38 (95%)	2.2	.147
Educational status	Primary = 26 (60.5%)	Primary= 0 (0%)		
	J. Secondary= 10 (23.3%)	J. Secondary = 6 (15%)	36.6	<.001*
	S.Secondary = 7 (16.3%)	S. Secondary = 8 (20%)		
	Tertiary =0 (0%)	Tertiary = 26 (65%)		
Social Habits				
Cigarette	Yes = 5 (11.6%)	Yes = 4 (10%)	0.3	.598
Smokers	No = 38 (88.4%)	No = 36 (90%)		
Alcohol	Yes = 9 (20.9%)	Yes = 14 (35%)		
	No = 34 (79.1%)	No = 26 (65%)	2.7	.097
Occupational				
Exposure to mixed	21.23 (1.9)+			
chemicals		-		
(years)				
Daily duration of work	10.50 (0.3)+			
in the mechanic				
village (Hours)				

#### Table 4. Comparison of demographic, social habits and occupational exposure to mixed chemicals between cases and controls

+=values in mean (SEM), X2 = Chi-square test, p = probability, \*= statistically significant, J. Secondary=junior secondary education, S.Secondary=senior secondary education

# Table 5. Regression of testosterone with oxidative stress biomarkers and occupational exposure to mixed chemicals with anthropometry in cases and controls

Index	Group	Predictors	Beta	Р
Testosterone (nmol/L)	Cases	(Constant)		
Adjusted R <sup>2</sup> = 8.6%		MDA(µmol/L)	0.13	.043*
R Square = 0.304				
p = 0.226				
		GST(µmol/ml)	-2.45	.049*
	Controls			
Adjusted $R^2 = 10.1\%$		SOD (U/ml)	-7.52	.02*
R Square = 0.210				
p = 0.056				
DOEMC (Years)	Cases	(Constant)		
Adjusted R <sup>2</sup> = 79.5%		BMI	-9.2	.048*
R Square = 0.844		WC	-0.8	.002*
p = 0.000		WHR	-0.5	.001*

β=Regression Coefficient, p=probability, MDA=Malondialdehyde, SOD=Superoxide dismutase, GST=Glutathione-S-transferase, BMI = Body mass Index, WC = Waist circumference, WHR=waist hip ratio, DOEMC=duration of occupational exposure to mixed chemicals

Regression of DOEMC with anthropometry in cases is also shown on Table 5. The regression was a good fit ( $R^2adj = 79.5\%$ ) and the overall relationship was significant (P< .001). DOEMC was negatively related with BMI ( $\beta$ = -9.2, P=.048), WC ( $\beta$ = -0.8, P=.002) and WHR ( $\beta$ = -0.5, P=.001).

#### 4. DISCUSSION

Oxidative stress is increasingly being recognized as a possible mechanism in the toxicity of various chemicals exposure at work places and in the aetiology of male infertility [47,48]. Recently, reduced TAC was observed in association with hypogonadism in males exposed to mixed chemicals in a mechanic community [32]. Similarly, other oxidative stress biomarkers, TPP and OSI in this present study were elevated in cases compared with controls. These indices were higher in eugonadic cases compared with controls. Furthermore, similar levels of these markers were observed between eugonadic and hypogonadic cases.

Our results suggest that oxidative stress as indicated by TPP and OSI may be due to occupational exposure to mixed chemicals in the cases irrespective of their gonadal status. A possible explanation for these observations is that available biomarkers measure different aspects of oxidative stress. TAC estimates both endogenous and exogenous antioxidants in a medium [15,16]. TPP quantifies all hydrogen peroxides and other derivatives of peroxides produced physiologically in the body [7]. While OSI is a combined measurement that shows the ratio of pro-oxidants and antioxidants [11].

Individual oxidative biomarkers may be important in assessing subtle alteration in antioxidant defence system [12]. Our present results also showed elevated MDA. H<sub>2</sub>O<sub>2</sub> and GST in exposed cases compared with controls as well as eugonadic cases compared with controls. Again, levels of these markers were similar in both eugonadic and hypogonadic exposed automechanics corroborating our earlier explanations. MDA results from free radical attack on polysaturated fatty acids of biological membranes, and used for monitoring lipid peroxidation in biological samples [13]. H<sub>2</sub>O<sub>2</sub> has the capacity to oxidize intracellular components directly and is could diffuse through cells and cross cell membranes, before decomposing to yield the highly reactive hydroxyl radical [49,50]. Glutathione-S-transferase (GST) is an antioxidant enzyme that catalyzes the conjugation of GSH, which acts as a nucleophile that binds with reactive electrophiles to prevent DNA damage. This activity detoxifies peroxidized lipids and enables the breakdown of xenobiotics [51]. Increase in lipid peroxidation due to chemical toxicity leading to alterations in the antioxidant defence system which normally protects against free radical toxicity has been reported [21].

However, SOD, GPX and GSH were similar in all comparisons between cases and controls, eugonadic cases and controls as well as eugonadic and hypogonadic cases in this study. It appears that the observed oxidative stress in the cases due to occupational exposure to mixed chemicals in this study may not relate to SOD, GPX or GSH depletion.

MDA is the final product of lipid peroxidation and widely used as an indication of tissue damage [52]. Increase in ROS and elevated MDA were demonstrated in male rats exposed to lead and cadmium acetate [53]. The toxic effects of mixed chemicals in the biological systems have been linked to increased lipid peroxidation (damage to lipid bilayer and DNA) and depletion of enzyme activities [54]. Enhanced production of ROS can damage biological membranes and other classes of macromolecules [55,56,57].

Testosterone regulates sexual behaviour and modulates every component involved in erectile function and reproduction [27,28]. It has been shown to have direct antioxidant effects by increasing the activities of antioxidant enzymes, thus contributing to antioxidant capacity [30,58]. Some studies have shown that increased TAC has been associated with successful fertilization [19,59]. The correlation total testosterone with TAC in male subjects has been shown [31]. In this present study, regression of testosterone with all oxidative stress biomarkers (including TAC) showed negative relationship with SOD in the controls. In the cases, relationship of testosterone with MDA was positive but negative with GST.

Testosterone is synthesized from cholesterol [34]. We postulate that this process involves the generation of ROS and depletion of antioxidant-SOD in eugonadic state in oxidative stress free environment/conditions. However, in oxidative stress conditions, there may be induction of GST [60]. and depletion of testosterone to detoxify peroxidised lipids resulting in MDA reduction[24]. Our findings probably reflect the contributory antioxidant role of testosterone in reducing the oxidative stress leading to hypogonadal state observed by others [30,32,59]. Oxidative stress is known to be present in individuals with metabolic syndrome (MS) which has abdominal obesity as one of its key components [61]. WC and WHR are measures of abdominal obesity, a key component of the MS, which has been implicated in the aetiology of hypogonadism [34,35]. Okoli et al. [32] also showed increased WC and WHR ratio with normal BMI as well as reduced testosterone levels and TAC in the auto mechanics occupationally exposed to mixed chemicals. It is therefore uncertain if the observed hypogonadism and oxidative stress was due to abdominal obesity present in these men or their occupational exposure to mixed chemicals.

In the cases, regression of DOEMC with anthropometry showed negative relationships with BMI, WC and WHR. These results suggest that both general (as represented by BMI) and abdominal obesity may not contribute to the oxidative stress observed in the cases in this present study. By virtue of their occupation, automechanics are physical active. Low education and enlightenment of the cases might have contributed to the oxidative stress observed in this study. Consumption of diets rich in fruits and vegetables enhance total antioxidant capacity and fight against lipid peroxidation [32,62]. Thus knowledge of diets rich in antioxidants may be beneficial to the cases.

#### 5. CONCLUSION

Oxidative stress was observed in automechanics occupationally exposed to mixed chemicals. The cases had alterations in some oxidative stress biomarkers. Biomarkers of increased lipid peroxidation-MDA, H<sub>2</sub>O<sub>2</sub>, TPP and OSI together with known enzymatic antioxidant, GST levels were elevated in cases than controls. However, other antioxidant enzymes, SOD, GPX and non-enzymatic antioxidant, GSH were similar between cases and controls. These findings appear unrelated to the gonadal status of the participants. Testosterone had negative relationship with SOD in controls only while its relationship with GST was negative but positive with MDA in cases only. Relationships of DOEMC with BMI (measure of general obesity), WC and WHR (measures of abdominal obesity) were negative. These observations are suggestive of the presence of oxidative stress in auto-mechanics exposed to mixed chemicals irrespective of their gonadal status and without the contribution of general or abdominal obesity. However, testosterone and GST may have antioxidant roles in reducing the observed lipid peroxidation in these men.

### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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