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

S. A. Adekola¹, M. A. Charles-Davies¹*, A. A. Onifade¹ and S. U. Okoli¹

¹Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

**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.

**Article Information**

DOI: 10.9734/BJMMR/2016/22350

Editor(s):
(1) Claudia Borza, Department of Pathophysiology, “Victor Babes” University of Medicine and Pharmacy, Romania.

Reviewers:
(1) Margarida Maria Esteves Florindo, Portuguese Red Cross Superior Health School, Portugal.
(2) Sema Kalkan Uçar, Ege University, Turkey.
(3) D. S. Sheriff, Benghazi University, Benghazi, Libya.

Complete Peer review History: http://sciencedomain.org/review-history/12469

**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/m² 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...
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$_2$O$_2$), 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$_2$O$_2$ 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 workplace 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$_2$O$_2$) 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 non-enzymatic 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 detoxifying 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 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$^2$ respectively (cases). They were age (38.7 (1.3) years; $P = 0.067$) and BMI matched (24.1 (2.8) Kg/m$^2$; $P = 0.654$) with 40 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°C 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 eugonad (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$_2$O$_2$, 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) µmol/L versus 1186 (44.6) µmol/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].

$$\text{OSI (in %)} = \frac{\text{TPP (µmol H}_2\text{O}_2)}{\text{TAC (µmol/L)}} \times 100$$

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 t-test was used to test the significant differences between mean values. Multiple regression analysis was employed to find relationships between the quantitative variables while Chi-square 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$_2$O$_2$ 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$_2$O$_2$, GST were significantly higher in eugonadic cases compared with controls ($P<0.05$).

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 ($P>0.05$).

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 ($P<0.001$). 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 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 ($P=0.226$) in the cases. However, testosterone was
positively related with MDA (β = 0.13, P = 0.043) and negatively related to GST (β = -2.45, P = 0.049). Similarly, the regression of testosterone with LH and oxidative biomarkers in controls was not a good fit (R² adjusted = 10.1%) and the overall relationship was not significant (P = 0.05). However, testosterone was negatively related with SOD (β = -7.52, P = 0.02).

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 (µmolH₂O₂/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* |
| H₂O₂ (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)   | .326   |

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

Table 2. Comparison of oxidative stress indices between eugonadic cases and controls

| Oxidative stress biomarkers | Eugonadic cases n = 30 | Controls n = 40 | P      |
|----------------------------|------------------------|-----------------|--------|
| TPP (µmolH₂O₂/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* |
| H₂O₂ (µmoles)              | 10.6 (3.1)             | 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, H₂O₂ = hydrogen peroxide, GST = glutathione-S-transferase, GSH = reduced glutathione

Table 3. Oxidative stress biomarkers between eugonadic and hypogonadotrophic hypogonadic cases

| Oxidative stress biomarkers | Eugonadic cases n = 30 | Hypogonadotrophic hypogonadic cases n = 6 | P      |
|----------------------------|------------------------|------------------------------------------|--------|
| TPP (µmolH₂O₂/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   |
| H₂O₂ (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, H₂O₂ = hydrogen peroxide, GST = glutathione-S-transferase, GSH = reduced glutathione
Table 4. Comparison of demographic, social habits and occupational exposure to mixed chemicals between cases and controls

| Demography          | Cases n=43 | Controls n=40 | X²  | P  |
|---------------------|------------|---------------|-----|----|
| Marital status      | Single = 6 (14%) | Single = 2 (5%) | 2.2 | .147 |
|                     | Married = 37 (86%) | Married = 38 (95%) |     |     |
| Educational status  | Primary = 26 (60.5%) | Primary = 0 (0%) | 36.6 | <.001* |
|                     | J. Secondary= 10 (23.3%) | J. Secondary = 6 (15%) |     |     |
|                     | 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   |             |               |     |     |
| chemicals           | 21.23 (1.9)+ | -             |     |     |
| (years)             |             |               |     |     |
| Daily duration of   |             |               |     |     |
| work in the mechanic | 10.50 (0.3)+ | -          |     |     |
| village (Hours)     |             |               |     |     |

+=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 | P  |
|---------------------|-------|------------|------|----|
| Testosterone (nmol/L)| Cases | (Constant) |      |    |
| Adjusted R² = 8.6%  |       | MDA(µmol/L) | 0.13 | .043* |
| R Square = 0.304    |       |            |      |    |
| p = 0.226           |       |            |      |    |
| Controls            |       | GST(µmol/ml) | -2.45 | .049* |
| R Square = 0.210    |       |            |      |    |
| p = 0.056           |       |            |      |    |
| DOEMC (Years)       | Cases | (Constant) |      |    |
| Adjusted R² = 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 (R²adj = 79.5%) and the overall relationship was significant (P< .001). DOEMC was negatively related with BMI (β= -9.2, P= .048), WC (β= -0.8, P= .002) and WHR (β= -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$_2$O$_2$ and GST in exposed cases compared with 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$_2$O$_2$ 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 total 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, auto-mechanics are physically 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 auto-mechanics occupationally exposed to mixed chemicals. The cases had alterations in some oxidative stress biomarkers. Biomarkers of increased lipid peroxidation-MDA, H$_2$O$_2$, 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.

REFERENCES

1. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility. Eur J Med. 2009;2:205–208.
2. Khalil AA, Hussein HM, Sarhan EM. Oxidative stress induces idiopathic infertility in Egyptian males. African Journal of Biotechnology. 2014;11:1516-1522.
3. Emopkae MA, Udia MA, Mohammed PO, Omale-Itodo AZ. Hormonal abnormalities in azospermic men in Kano, Northern Nigeria. India J Med Res. 2006;124:299-304.
4. Aitken RJ, Krausz C. Oxidative stress, DNA damage and the chromosome. Reproduction. 2001;122:497–506.
5. Ramsey. Medical management guidelines for acute chemical exposure; Agency for Toxic substances and Disease Registry (NSDR); 1992.
6. Anetor JI. An evaluation of the nutritional, metabolic and immune status in occupational lead toxicity. PhD Thesis University of Ibadan; 1997.
7. Koracevic DG, Koracevic V, Ojordjevic S, Andrejevic VC. Method for the measurement of antioxidant activity in human fluid. J. Clin Pathol. 2001;54:356-361.
8. Cross CE, Halliwell B, Borish ET. Oxygen radicals and human disease. Ann Intern Med. 1987;526-545.
9. Porter NA, Mills KA, Caldwell SE. Mechanism of free radical oxidation of unsaturated lipids. Lipids. 1995;30:277-290.
10. Halliwell B. Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. Free Rad. Res. 1996;25:57-74.
11. Abuelo A. Oxidative stress index as a new tool to assess redox status in dairy cattle during the transition period. E Pub. 2013; 7(8):1374-1378.
12. McCord JM, Day ED Jr. The deoxyribose assay: An assay for both free hydroxyl radical and for site-specific hydroxyl radical production. FEBBS Lett. 1978;66:139-142.
13. Dezwart LL, Meerman JN, Commandeur ML. Biomarkers of free radical damage application in experimental animals and in humans. Free Radic Biol Med. 1999;26:202-226.
14. Halliwell B. Free radicals and antioxidants: A personal view. Nutr Rev. 1994;1:253-265.
15. Rezaie A, Parker RD, Abdullahi M. Oxidative stress and pathogenesis of inflammatory bowel disease: An
epiphenomenon or the cause. Dis. Sci. 2007;52:2015-2021.

16. Krajcir N, Chowdary H, Gupta S, Agarwal A. Female infertility and assisted reproduction: Impact of oxidative stress. Current women’s Health Reviews. 2008;4: 9–159.

17. Wang HY, Xin Z, Yang H, Ganea D. Vasoactive intestinal peptides inhibits IL-4 production in Murine T cells by a post translational mechanism. J. Immuno. 1997;156:3243-3253.

18. Lewis SE, Boyle PM, McKinney KA, Young IS, Thompson W. Total antioxidant capacity of seminal plasma is different in fertile and infertile men. FertilSteril. 1995;64:868–870.

19. Paszkowicki T, Clarke RN. Antioxidant capacity of preimplantation embryo culture medium declines following the incubation of poor quality embryos. Human Reprod. 1996;11:2493-2495.

20. Patra RC, Swarup D, Senapat SK. Effects of cadmium on lipid peroxides and superoxide dismutase in hepatic, renal and testicular tissue of rats. Veterinary and Human Toxicology. 1999;41(2):65–67.

21. Pasqualotto FF, Lucon AM, Sobreiro BP, Pasqualotto E, Arap S. Effects of medical therapy, alcohol, smoking, and endocrine disruptors on male infertility. Rev Hosp Clin Fac Med Sao Paulo. 2004;59:375-382.

22. Fridovich I. Biological effects of the superoxide radical. Archives of Biochemical and Biophysicsology. 1986;247: 1-11.

23. Pompella A. The changing faces of glutathione, a cellular protagonist. Biochem. Pharmacol. 2003;66:1499-1503.

24. Oakley A. GSTs: A structural perspective. Drug Metab. Rev. 2011;43(2):138-51.

25. Laloraya M, Jain S, Thomas M, Kaspergaoonkar S, Pradeep Kumar G. Estrogen surge: A regulatory switch for superoxide radical generation at implantation. Biochem MolBiol Int. 1996; 39:933-940.

26. Massafrca C, De Felice C, Gioia D, Buonocore G. Variation in erythrocyte antioxidant glutathione peroxidase activity during the menstrual cycle. Clin Endocrinol. 1998;49:63-67.

27. Ing JJ. Steroid hormones regulate gene expression post-transcriptionally by altering the stabilities of messenger RNAs. Biol Reprod. 2005;72:1290-1296.

28. Andrea MI, Burat J, Corona G, Goldstein I, et al. A critical analysis of the role of testosterone in erectile function: From pathophysiology to treatment – A systemic review. J. Eururo. 2013;2838(13):00876-882.

29. Gould DC, Petty R. The male menopause: Does it exist? for: Some men need investigation and testosterone treatment. West. J. Med. 2000;173(2):76–78.

30. Massafa C, Gioia D, De Felice C, picciolini E, De Leo V, Bonifazi M, Bernabei A. Effects of estrogens and androgens on erythrocyte antioxidant superoxide dismutase, catalase and glutathione peroxide activities during the menstrual cycle. J Endocrinol. 2000;167:447-452.

31. Macini A, Festa R, Sihestrini A, Mcolotti N, Di Donna V, La Tovre G, Pontecorri A, Meucci E. Hormonal regulation of total antioxidant capacity in seminal plasma. Journal of Andrology. 2010;30(5):534-546.

32. Okoli SU, Charles-Davies MA, Onifade AA, Adekola S. Hypogonadism in males exposed to mixed chemicals in a mechanic village in Bodija, Ibadan. JSRR. 2015;8(7): 1-9.

33. Umoh U. Charles-Davies MA, Adeleye J. Serum testosterone and lipids in relation to sexual dysfunction in males with metabolic syndrome and type2 diabetes mellitus. International Journal of Medicine and Medical Sciences. 2010;2:402-412.

34. Fabian UA, Charles-Davies MA, Fasanmade AA, Oyewole OE, Owolabi MO, Adebusuyi JR, Hassan O, Ajobo MT, Ebesunun MO, Adigun K, Akinlade KS, Arinola OG, Agbedana EO. Male sexual dysfunction, leptin, pituitary and gonadal hormones in nigerian males with metabolic syndrome and type 2 diabetes mellitus. Journal of Reproduction and Infertility; 2015. (In press).

35. Charles-Davies MA, Arinola OG, Fasanmade AA, Oyewole OE, Owolabi MO, Adebusuyi JR, Hassan O, Ajobo MT, Ebesunun MO, Adigun K, Akinlade KS, Fabian UA, Popoola OO, Rahamon SK, Okunbolade W, Ogunlakin MA, Agbedana EO. Indices of metabolic syndrome in 534 apparently healthy traders in a local market in Ibadan, Nigeria. Journal of US-China Medical Science. 2012;9(2):91–100.

36. Benzie FF, Strain JJ. Ferric reducing/antioxidant power assay: Direct measure
Adekola et al.; BJMMR, 12(9): 1-11, 2016; Article no.BJMMR.22350

37. Miyazawa T. Determination of phospholipids hydroperoxides in human blood plasma by a chemiluminescence-HPLC assay. Free Radic. Biol. Med. 1989; 7:209-217.

38. Harma M, Harma M, Erel O. Measurement of the total antioxidant response in preeclampsia with a novel automated method. Eur J. Obstet. Gynaecol. Reprod. Biol. 2005;10:47-51.

39. Yeni E, Gulum M, Selek S. Comparison of oxidative/antioxidative status of penile corpus cavernosum blood and peripheral venous blood. Int. J. Impot. Res. 2005;17:19-22.

40. Harma M, Harma M, Erel O. Increased oxidative stress in patients with hydatiform mole. Swiss Med. Weekly. 2003;133:563-566.

41. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG. Selenium: Biochemical role as a component of glutathione peroxidase. Science. 1973;179:588-590.

42. Wolff H. Methods for the detection of male genital tract inflammation. Andrologia. 1998;30(1):35-39.

43. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J BiolChem. 1972; 247:3170-3175.

44. Habig H, Pabst G, Jakoby B. Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 1974;249:7130-7139.

45. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J. Lab. Clin. Med. 1963;61:882-888.

46. Adam-Vizi V, Seregi M. Receptor dependent stimulatory effect of noradrenaline on Na⁺/K⁺ ATPase in rat brain homogenate. Role of lipid peroxidation. Biochem. Pharmacol. 1982; 31:2231-2236.

47. Aitken RJ, Buckingham DW, West K. Differential contribution of leukocytes and spermatozoa to the generation of reactive oxygen species in the ejaculates of oligospermic patients and fertile donors. J. Reprod. Fertil. 1992;94:451-462.

48. Rim KT, Se-wook S, Hyeon-Treong. Oxidative DNA damage from nanoparticle exposure and its application to workers health: A literature review. Safe Health Work. 2013;4(4):177-186.

49. Hyslop PA, Hinshaw DW, Hasley WA (Jr). Mechanism of oxidation mediated cell injury. J. Biochem. 1998;263:1665-755.

50. Halliwell B, Gutteridge MC. Role of free radicals in catalytic metal in human disease. Method enzyme. 1990;1861:1-85.

51. Miller JA, Miller EC. Ultimate chemical carcinogen as reactive mutagenic electrophiles. In: Hiat HH, Watson JA, Winsten JA (eds). Origin of human cancer. Cold spring habor Laboratory, cold spring habor NY. 1977;605-627.

52. Dhir B, Sharmila P, Saradhi PP. Hydrophytes lack potential to exhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline. Aquat. Toxicol. 2004;66:141-147.

53. Pandya C, Pillai P, Nampoothiri LP, Bhatt N, Gupta S. Effect of lead and cadmium co-exposure on testicular steroid metabolism and antioxidant system of adult male rats. Andrologia. 2012; 44(Suppl 1):813-22. DOI: 10.1111/j.1439-0272.2010.01137.x Epub 2011 Sep 20.

54. Stohs SJ. The role of free radicals in toxicity and disease. Journal of Basic Clinical Physiology and Pharmacology. 1995;6:205-228.

55. Mittler R. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 2002;7:405-410.

56. Blikhın O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: A review. Ann. Bot. 2003;91:179-194.

57. Andrade SAL, Gratão PL, Azevedo RA, Silveira APD, Schiavinato MA, Mazzaferra P. Biochemical and physiological changes in jackbean under mycorrhizal symbiosis growing in soil with increasing Cu concentrations. Environ. Exp. Bot. 2010; 68:198-207.

58. Demirbag R, Yilmaz R, Erel O. The association of total antioxidant capacity with sex hormones. Scand. Cardiovasc J. 2005;39:12-176.

59. Lewis SE, Boyle PM, McKinney KA, Young IS, Thompson W. Total antioxidant capacity of seminal plasma is different in
fertile and infertile men. Fertil Steril. 1995;64:868–870.

60. Veal EA, Toone WM, Jones N, Morgan BA. Distinct roles for glutathione S-transferases in the oxidative stress response in Schizosaccharomyces pombe. The Journal of Biological Chemistry. 2002;277:35523–35531.

61. Rahamon SK, Arinola OG, Charles-Davies MA, Akinlade KS, Fasanmade AA, Olaniyi JA, Oyewole OE, Owolabi MO, Adebusuyi JR, Hassan O, Ajobo MT, Ebesunun MO, Adigun K, Fabian UA, Popoola OO, Okunbolade W, Agbedana EO. Impact of dietary intervention on selected biochemical indices of inflammation and oxidative stress in Nigerians with metabolic syndrome. European Journal of Nutrition and Safety. 2014;4:137-149.

62. Carlos K, Bucalen F. Total antioxidant capacity; a biomarker in biomedical and biomedical and nutritional studies. Journal of Cell and Molecular Biology. 2008;7:1–15.