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Relationship between Testosterone, Oxidative Stress Biomarkers and Antioxidant levels in Male Auto-mechanics in Ibadan, Nigeria

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ABSTRACT

Hypogonadism attributable to males with metabolic syndrome was observed in automechanics occupationally exposed to mixed chemicals accompanied by oxidative stress (OS). We evaluated association between testosterone, OS biomarkers, enzymatic and non-enzymatic antioxidants in normal weight automechanics in Ibadan. This was a prospective cross sectional study involving 100 normal weight males aged 18 – 60 years. They were 50 automechanics in Ibadan, age and anthropometry matched with 50 eugonadic males from University College Hospital and environs (controls). Demographic, anthropometry, social habits and dietary history were obtained by standard methods. Blood (10mL) was collected and serum/plasma was used for biochemical analyses. Enzymatic antioxidants (catalase, glutathione peroxidase, superoxide dismutase (SOD) and glutathione -S- transferase (GST); non-enzymatic antioxidants (reduced glutathione (GSH), selenium and zinc), OS biomarkers (hydrogen peroxide (H₂O₂), malondialdehyde (MDA), total antioxidant capacity (TAC), total plasma peroxides (TPP) and oxidative Stress index (OSI) were estimated spectrophotometrically. Testosterone was assayed by enzyme immunoassay method (Dialab, Austria). Student's t-test, Chi-square test and multiple regression were used for comparisons, associations and relationships respectively, which were significant at $P<0.05$. Testosterone, TPP, OSI, GST, MDA, H₂O₂, selenium and zinc concentrations were significantly higher while catalase and SOD concentrations were lower in automechanics than controls ($P<0.05$). However, testosterone levels in both groups were within the normal reference interval. TAC, OSI and GSH had significantly negative relationship while TPP had positive relationship with years at occupation in automechanics only ($P<0.05$). Automechanics may have OS but not hypogonadism probably due to increased antioxidant intake.

Keywords: *antioxidants, leydig cell dysfunction, oxidative stress, testosterone*

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INTRODUCTION

The prevalence of infertility among married couples in African countries ranges from 15% to 30%. About 30% of infertility is due to male factor (Umeora *et al.*, 2007). Exposure to mixed chemicals in work place leads to generation of free radicals which if unaccompanied by available antioxidant leads to oxidative stress (Anetor and Adeniyi, 2001; Flora *et al.*, 2008). Oxidative stress (OS) contributes to many pathological conditions including male infertility (Agarwal *et al.*, 2014). It is a consequence of an imbalance between the production of ROS and the body's antioxidant defence mechanisms (Agarwal and Prabakaran, 2005). The resulting damage to cells and organs may activate and/or accelerate disease processes (Basu, 2010).

Chemical toxicity is considered a great threat in rapidly industrializing countries (Anetor *et al.*, 2009). Automechanics are usually prone to long term chemical toxicity due to their occupation as they are exposed to mixed chemicals through contact with their skin and sometimes through the oral route. These toxic substances can induce oxidative stress (OS) through their capacity to interact with Reactive Oxygen Species (ROS), thereby increasing their oxidant activity or affecting membrane integrity (Oteiza *et al.*, 2004).

Reactive oxygen species are produced by living organisms as a result of normal cellular metabolism. At low to moderate concentrations, they function in physiological cell processes, but at high concentrations, they produce adverse modifications to cell components, such as lipids, proteins, and DNA (Valko *et al.*, 2006; Stadtman, 2004). ROS contain two

unpaired electrons in the outer shell. These include superoxide anion (O_2^-), hydroxyl radical (OH^\cdot), singlet oxygen (1O_2), and hydrogen peroxide (H_2O_2). Superoxide anions are formed when oxygen (O_2) acquires an additional electron, leaving the molecule with only one unpaired electron. Superoxide anion is continuously formed within the mitochondria. The rate of formation depends on the amount of oxygen flowing through the mitochondria at any given time.

Hydrogen peroxide produced *in vivo* can either be converted to the highly damaging hydroxyl radical or catalyzed and excreted as water (Goldfarb, 1999). The principal problem is that H_2O_2 easily crosses cellular membranes while receiving one more electron, normally originating from iron or copper resulting in hydroxyl radical, which needs only one more electron to be stabilized. Hydroxyl radicals are short-lived, but are the most damaging radicals within the body. This type of free radical can be formed from $O_2^{\cdot -}$ and H_2O_2 via the Harber-Weiss reaction ($O_2^{\cdot -} + H_2O_2 \rightarrow OH^\cdot + OH^- + O_2$) (Kanti das *et al.*, 2015). Cells living under aerobic conditions constantly face the oxygen (O_2) paradox: O_2 is required to support life, but its metabolites such as ROS can modify cell functions, endanger cell survival, or both (Ashok and Ali, 2003).

Antioxidants are substances that may protect cells from the damage caused by free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. There are two types of antioxidants: enzymatic and non-enzymatic antioxidants. Enzymatic antioxidants are also known as natural antioxidants. They act by neutralizing excessive ROS, and preventing it from damaging the cellular structure. They include catalase, glutathione peroxidase, glutathione-s-transferase and superoxide dismutase.

Catalase detoxifies both intracellular and extracellular H_2O_2 to water and oxygen (Baker *et al.*, 1996). Glutathione peroxidase protects against lipid peroxidation of plasma membrane of spermatozoa. It scavenges lipid peroxides thereby arresting the progressive chain reaction of lipid peroxidation. It also scavenges H_2O_2 , which is responsible for the initiation of lipid peroxidation. Glutathione-s-transferase catalyses the conjugation of glutathione and detoxifies peroxidised lipid (Adekola *et al.*, 2016). Superoxide dismutase (SOD) scavenges both extracellular and intracellular superoxide anion and prevents lipid peroxidation of the plasma membrane. In order to act against H_2O_2 , SOD must be conjugated with catalase or glutathione peroxidase (Jeulin *et al.*, 1989). SOD also prevents premature hyperactivation and capacitation induced by superoxide radicals before ejaculation (De Lamirande and Gagnon, 1995).

Non-enzymatic antioxidants are also known as synthetic antioxidants or dietary supplements (Agarwal and Said, 2005). The body's complex antioxidants system is influenced by dietary intake of antioxidant vitamins (such as vitamin C, vitamin E), glutathione, beta carotene, minerals (such as selenium and zinc) (Pierce *et al.*, 2004). Glutathione is produced by the body, but levels of this antioxidant decline with age (Packer, 1999). Glutathione deficiency can lead to instability of the mid-piece, resulting in defective motility (Lenzi *et al.*, 2004). Beta-carotene is able to quench singlet oxygen without degradation and reacts with free radicals, such as peroxy, hydroxyl and superoxide radicals. Carotenoids

have consistently been shown to prevent or decrease oxidative damage to DNA, lipids and proteins (Agarwal *et al.*, 2012; Chapman, 2012). Selenium research has attracted tremendous interest because of its important role in antioxidant selenoproteins for protection against oxidative stress initiated by excess ROS (Tapiero *et al.*, 2003). Zinc may protect protein sulfhydryl groups from oxidative modification by influencing the conformation and reducing potential of thiol groups. It can also antagonize the activity of transition metals such as iron and copper by competing with them, thereby preventing transition metal mediated oxidative modifications (Zago and Oteiza, 2001).

Malondialdehyde (MDA) is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation (Janero, 1990). MDA can combine with several functional groups on molecules including proteins, lipoproteins, RNA and DNA (Sevilla *et al.*, 1997). Quantification of lipid peroxidation by measuring MDA levels is essential to access the role of oxidative injury in pathophysiological disorders (Halliwell, 1995).

Total antioxidant capacity (TAC) measures the antioxidant capacity of all antioxidants in a biological sample, and reflects the antioxidant status of the plasma (Ferari, 2001; Erel, 2004). Measuring TAC can provide information on an individual's overall antioxidant status, which may include those antioxidants not yet recognized or not easily measured (Lamont *et al.*, 1997). Total Plasma peroxide (TPP) measures all hydrogen peroxide and other derivatives of peroxides produced physiologically in the body that diffuse into plasma (Koracevic *et al.*, 2001). oxidative stress index (OSI), an indicator of the degree of oxidative stress (Harma *et al.*, 2003).

Testosterone is the male hormone synthesized from cholesterol. Produced in leydig cells, it plays an essential role in the development of the male reproductive phenotype (Ge and Hardy, 2007). Obesity may induce systemic oxidative stress (Esposito, 2006; Fonseca *et al.*, 2007). Okoli *et al.* (2015) showed association of hypogonadism with OS in automechanics. Fat tissue accumulation in men has also been reported to also lower serum levels of testosterone (Fabian *et al.*, 2016). Testosterone has been shown to increase the effects of antioxidant enzymes, such as glutathione peroxidase (Massafra *et al.*, 2000). Induction of GST and depletion of testosterone for probable detoxification in oxidative stress conditions have been reported (Adekola *et al.*, 2016). This study is therefore aimed at evaluating the relationship between antioxidants (enzymatic and non-enzymatic) and testosterone in normal weight automechanics in mechanic communities in Ibadan.

MATERIALS AND METHODS

Study Design: This is a prospective cross-sectional study conducted on normal weight male automechanics occupationally exposed to mixed chemicals in Ibadan. Ethical approval was obtained from the University of Ibadan/University College Hospital, Ibadan (UCH) Joint Ethical Review Committee (NHREC/05/0118).

Study Population: A total of 100 male participants with normal BMI and no component of MS aged 18 – 60 years

enrolled for this study. 50 male automechanics were recruited from different mechanic communities in Ibadan-Dandaru (9 participants), Ring Road (14 participants), Bodija (11 participants), Mokola (10 participants) and Alalubosa (6 participants). They were age and anthropometry matched with 50 apparently healthy males-students and staff of the UCH after informed consent. All participants were not on any medication/antioxidant supplements. Those with chronic disease conditions-diabetes mellitus, cardiovascular disease or any form of neurodegenerative disease were excluded.

Body Mass Index (BMI): All participants had normal weight BMI (18.5 – 24.9 Kg/m²) according to World Health Organization (WHO), 2009 classification

Metabolic Syndrome (MS) Criteria: The Joint interim criteria (Alberti *et al.*, 2009) for the clinical diagnosis of MS were used. The participants had none of the MS components, which are elevated waist circumference (≥ 94 cm), elevated triglycerides (≥ 150 mg/dL), reduced HDLC (< 40 mg/dL), elevated blood pressure (systolic ≥ 130 mmHg, diastolic ≥ 85 mmHg), elevated plasma fasting glucose (FPG) (≥ 100 mg/dL).

Demography, Social Habits and Dietary History: Demography (age, educational status, marital status), social habits (smoking history, alcohol consumption) and dietary history (dairy product intake, fruit and vegetable intake) were obtained through semi structured pretest questionnaire administered to study participants.

Blood Pressure and Anthropometry: Blood pressure and anthropometry-body weight, height, BMI, waist and hip circumferences, waist-hip ratio and waist-height ratio were obtained from the participants by methods described by Umoh *et al.* (2010); Charles-Davies *et al.* (2012).

Blood Collection: Ten mL of fasting venous blood was obtained from all study participants. 4 mL were put in plain tubes and allowed to clot for 30 minutes to obtain serum for the estimation of testosterone, antioxidants and oxidative stress biomarkers. 2 mL was put in a tube containing fluoride oxalate for glucose estimation while the remaining 4 mL was put in a tube containing Potassium EDTA (K₃EDTA) for lipid estimation. Plasma/serum obtained after centrifuging at 500g for 5 minutes were stored at -20°C until analyses.

Biochemical Indices

Testosterone: Testosterone was analyzed by enzyme-linked immunosorbent assay by method of Uotila (1981) using commercial kit (Dialab Austria).

Enzymatic Antioxidants: Catalase activity was determined according to the method of Asru and Sinha (1972). GPx was measured by method of Rotruck (1973). GST activity was determined according to Habig *et al.* (1974). SOD activity was assayed according to the method of Misra and Fridovich (1972).

Non-enzymatic Antioxidants: The method of Beutler *et al.* (1963) was used to estimate the level of reduced glutathione (GSH). Selenium and zinc were determined by flame atomic absorption spectrophotometer using a direct method as described by Kaneko (1999) (Buck Scientific Model 210/211 VGP, Germany).

Oxidative Stress Biomarkers: H₂O₂ was determined using the method of Wolf (1994). Estimation of MDA was carried out according to the method of Adam-Vizi and Seregi (1982). Measurement of the TAC was carried out using ferric reducing antioxidant power (FRAP) assay of Benzie and Strain (1999). TPP levels were determined using the ferrous oxidation (FOX2) method (Miyazawa, 1989) with minor modifications (Hamar *et al.*, 2005). OSI is the ratio of TPP levels to TAC and calculated as OSI (in %) = [TPP (μ mol H₂O₂)/TAC (μ mol/L)] $\times 100$.

Glucose and Lipids: Estimation of FPG was done by glucose oxidase method as described by Sacks *et al.* (2002). Estimation of triglyceride was done as described by Guder *et al.*, (2001) using commercial kit while estimation of HDLC was done as described by Burtis and Ashwood (1994) using commercial kits (Dialab, Austria).

Statistical Analysis: Statistical Package for the Social Sciences (SPSS) 20.0 was used to analyze data. Student's t test was used for comparison of quantitative variables. Multiple regression analysis was used to find relationships between the quantitative variable while chi-square test was used to find associations. Data obtained were significant at $P < 0.05$.

RESULTS

Demography, Social Habits and Dietary History in Automechanics and Controls: Table 1 compares the demographic, social habits and dietary history of automechanics with controls. There were no significant differences in mean age, and marital status of auto mechanics compared with controls ($P > 0.304$). Educational status, dairy products, fruits and vegetable intake and refined carbohydrate consumption were significantly higher in auto mechanics compared with controls ($P < 0.001$). Duration at work and years spent at occupation was significantly higher in automechanics compared with controls ($P < 0.001$).

Metabolic Syndrome Indices and Anthropometric Measurements in Automechanics and Controls: Table 2 compares metabolic syndrome indices and anthropometric measurements in automechanics and controls. There were no significant differences in metabolic syndrome components and anthropometric measures in auto mechanics and controls ($P > 0.065$).

Testosterone, Enzymatic, Non-Enzymatic Antioxidants, and Oxidative Stress Biomarkers in Automechanics and Controls: Table 3 compares testosterone, enzymatic, non-enzymatic antioxidants and oxidative stress biomarkers. Testosterone was significantly higher in automechanics compared with controls ($P < 0.001$).

Although, the levels in both groups were within normal reference interval, the enzymatic antioxidant catalase was significantly higher in controls compared with auto mechanics ($P < 0.003$), while GST and SOD were significantly higher in auto mechanics compared with controls ($P < 0.006$). There was no significant difference observed in GPx in auto-mechanics compared with controls ($P > 0.101$). The non-enzymatic antioxidant selenium and zinc were significantly higher in

auto-mechanics compared with controls ($P < 0.002$) while GSH showed no significant difference in auto mechanics and controls ($P > 0.209$). The biomarkers markers of oxidative stress, H_2O_2 , MDA, TPP and OSI were significantly higher in auto mechanics than the controls ($P < 0.001$) while TAC was significantly higher in controls compared with the automechanics ($P < 0.001$).

Table 1.
Demography, social habits and dietary history in automechanics and controls.

Qualitative Variables	Automechanics n = 50	Controls n = 50	X ²	P
Age (Years)	40.8(8.3)	39.1(7.9)	1.033 ⁺	0.304
Marital status	Single = 3(6%) Married = 47(94%)	Single = 5(10%) Married = 45(90%)	0.543	0.461
Educational status	NFE = 4(8%) PSLC = 13(26%) JSC = 5(10%) SSC = 25(50%) Graduate = 3(6%) PG = 0(0%)	NFE = 0(0%) PSLC = 1(2%) JSC = 0(0%) SSC = 2(4%) Graduate = 34(68%) PG = 13(26%)	77.8	<0.001*
Dairy products	Daily = 10(20%) Weekly = 9(18%) Occasionally = 29(58%) Never = 2(4%)	Daily = 7(14%) Weekly = 25(50%) Occasionally = 17(34%) Never = 1(2%)	11.5	0.009*
Vegetable/fruits	Daily = 21(42%) Weekly = 16(32%) Occasionally = 13(26%)	Daily = 6(12%) Weekly = 32(64%) Occasionally = 12(24%)	13.7	0.001*
Refined carbohydrate	Daily = 8(16%) Weekly = 7(14%) Occasionally = 30(60%) Never = 5(10%)	Daily = 10(20%) Weekly = 17(34%) Occasionally = 23(46%) Never = 0(0%)	10.6	0.014*
Years at occupation	<2 years = 0(0%) 2– 5 years = 0(0%) 6 – 9 years = 3(6%) ≥10 years = 47(94%)	<2 years = 4(8%) 2– 5 years = 31(62%) 6 – 9 years = 13(26%) ≥10 years = 2(4%)	16.821	<0.001*
Hours of work	9.2(2.1)	7.8(1.4)	3.794 ⁺	<0.001*
Days of work	5(0.7)	6(0.4)	6.98	<0.001*

X² = Chi-square. + = Student t-test used. p = probability. * = statistically significant. NFE = No formal education. PSLC = primary school leaving certificate. JSC = junior school certificate. SSC = senior school certificate. PG = Post Graduate., values in mean ± S.D.

Table 2:
Metabolic Syndrome Indices and Anthropometric Measurements in Automechanics and Controls.

Index	Automechanics (n = 50)	Controls (n = 50)	t	P
MS indices				
SBP (mmHg)	115.38(9.1)	114.76(6.1)	0.690	0.400
DBP (mmHg)	76.66(11.3)	76.60(10.8)	0.978	0.600
Glucose (mg/dL)	88.08(13.7)	89.78(14.3)	-1.700	0.544
Triglyceride(mg/dL)	118.62(10.5)	117.96(9.8)	0.660	0.746
HDL-C (mg/dL)	40.98(8.9)	42.44(8.5)	-1.460	0.402
WC (cm)	79.05(5.8)	76.71(6.7)	1.869	0.065
Anthropometry				
Weight (kg)	62.87(6.0)	64.33(6.4)	-1.175	0.243
Height (m)	1.73(0.1)	1.73(0.1)	-0.422	0.674
BMI (kg/m ²)	21.14(1.8)	21.47(1.7)	-9.22	0.359
HC (cm)	91991.90(6.2)	89.52(7.7)	1.703	0.092
WHR	0.86(0.3)	0.85(0.4)	1.265	0.209
WHtR	0.46(0)	0.44(0)	2.262	0.09

MS = Metabolic Syndrome. SBP = Systolic Blood Pressure. DBP = Diastolic Blood Pressure. HDL-C = High Density Lipoprotein – Cholesterol. BMI = Basal Mass Index. WC = Waist Circumference. HC = Hip Circumference. WHR = Waist to Hip Ratio. WHtR = Waist to Height Ratio. Values in Mean ± S.D. * = statistically significant.

Table 3:

Testosterone, Enzymatic, Non-enzymatic Antioxidants, and Oxidative Stress Biomarkers in Automechanics and Controls.

Index	Automechanics (n=50)	Controls (n= 50)	t	P
Testosterone (ng/mL)	8.79(2.5)	6.92(2.4)	3.759	<0.001*
Enzymatic Antioxidants				
Catalase (U/mL)	20.30(1.7)	21.10(1.3)	-3.045	0.003*
GPX (U/mL)	13.05(1.1)	12.64(1.4)	1.656	0.101
GST (µmole/mL)	1.42(0.4)	1.03(0.2)	6.648	<0.001*
SOD (U/mL)	1.60(0.5)	1.32(0.5)	2.818	0.006*
Non-enzymatic Antioxidants				
GSH (Ug/mL)	12.93(1.4)	12.64(0.8)	1.267	0.209
Se (µg/dL)	0.05(0.0)	0.04(0.0)	3.163	0.002*
Zn (µg/dL)	130.16(20.5)	116.36(23)	3.169	0.002*
Other Oxidative Stress Biomarkers				
H₂O₂ (µmoles)	2.79(0.7)	1.16(0.8)	10.667	<0.001*
MDA (µmole/L)	19.14(0.8)	14.89(1.0)	23.790	<0.001*
TAC (µmole/L)	866.71(29.9)	1086.11(53.6)	-25.296	<0.001*
TPP (µmoleH₂O₂/L)	12.23(0.9)	9.13(1.2)96	14.578	<0.001*
OSI(%)	1.41(0.1)	0.74(0.1)	30.567	<0.001*

H₂O₂ = Hydrogen Peroxide. MDA = Malondialdehyde. GPX = Glutathione Peroxidase. GST = Glutathione-S-Transferase. TAC = Total Antioxidant Capacity. TPP = Total Plasma Peroxide. OSI = Oxidative Stress Index. SOD= Superoxide Dismutase. GSH = Reduced Glutathione. Se = Selenium. Zn = Zinc. Values in Mean ± S.D. * = statistically significant.

Multiple Regression of Testosterone and Indices of Occupational Exposure to Chemical Toxicity with Oxidative Stress Biomarkers in Automechanics and Control:

Multiple regression showed no significant relationship between testosterone and oxidative stress biomarkers in automechanics and controls ($R^2 = 0.339$; $F =$; $P = 0.140$; $R^2 = 0.358$, $F =$, $P = 0.102$ respectively). However, TAC, GSH and OSI showed significantly negative relationships ($\beta = -4.656$, $F =$, $P = 0.043$; $\beta = -0.390$, $F =$, $P = 0.046$; $\beta = -10.252$, $P = 0.047$) respectively while TPP showed significantly positive relationship ($\beta = 9.954$, $F =$, $P = 0.046$) with years at occupation in automechanics but not in controls ($R^2 = 0.358$, $F =$, $P = 0.436$). No significant relationships were observed in number of hours and days at work with oxidative stress biomarkers in both automechanics ($R^2 = 0.240$, $F =$, $P = 0.585$; $R^2 = 0.371$, $F =$, $P = 0.585$) and controls ($R^2 = 0.281$, $P = 0.405$; $R^2 = 0.129$, $F =$, $P = 0.957$.) respectively.

DISCUSSION

Infertility is a problem of public health importance in Nigeria and many other developing nations because of its serious social implications on affected couples and families (Umeora *et al.*, 2007). Auto mechanics are exposed to residual used gasoline engine oils that accumulate on automobile parts, tools and workbenches. These chemicals are absorbed through the skin and can predispose an individual to OS and subsequent infertility. The impact of OS on fertility status has been extensively studied in recent years (Makker *et al.*, 2009). Observations in this study suggest that oxidative stress mechanisms may underlie the occupational exposure of automechanics to mixed chemicals as reported earlier by Okoli *et al.* (2015) and Adekola *et al.* (2016). This may be

responsible for the alterations observed in antioxidant enzymes status in this study.

Oxidative Stress index is an indicator of the degree of oxidative stress (Fiers *et al.*, 1999). It was significantly elevated in automechanics compared with the controls ($P < 0.05$). Quantification of lipid peroxidation by measuring MDA levels is essential to access the role of oxidative injury in pathophysiological disorders (Halliwell, 1996). A significant increase in MDA was observed in automechanics compared with controls ($P < 0.05$). SOD eliminates ROS by reducing superoxide to form hydrogen peroxide (Adekola *et al.* 2015). Automechanics had significantly raised SOD levels compared with controls ($P < 0.05$). H₂O₂ and TPP were significantly elevated in automechanics compared with controls ($P < 0.05$). TPP sums up all H₂O₂ and other derivatives of peroxides produced physiologically in the body that diffuse into plasma (Koracevic *et al.*, 2001).

Catalase catalyses the reaction of H₂O₂ into water and molecular oxygen (Paravicini and Touyz, 2008). A significant increase in catalase was observed in the controls compared with the automechanics ($P < 0.05$). Catalase detoxifies both intracellular and extracellular H₂O₂ to water and oxygen (Baker *et al.*, 1996). The reduced catalase observed in automechanics might be due to increase use of catalase in counteracting free radicals. GST is a family of phase II xenobiotic detoxifying enzymes. It was significantly raised in automechanics compared with controls ($P < 0.05$). Induction of GST in the presence of altered OS biomarkers in automechanics has been reported previously (Adekola *et al.*, 2016).

However, non-enzymatic antioxidants zinc and selenium were significantly raised in automechanics compared with controls ($p < 0.05$). Zinc may protect protein sulfhydryl groups from oxidative modification by influencing the conformation and reducing potential of thiol groups. It can also antagonize

the activity of transition metals such as iron and copper by competing with them, thereby preventing transition metal mediated oxidative modifications (Zago and Oteiza, 2001). Selenium plays important role in antioxidant selenoproteins for protection against oxidative stress initiated by excess ROS (Tapiero *et al.*, 2003). Consumption of vegetables and fruit were significantly higher in the auto mechanics and this might be the possible explanation for the increased selenium and zinc levels. GSH is a potent antioxidant with high redox potential and it also serves as a co-factor for several oxidative stress detoxifying enzymes (Valko, 2006). There was no significant difference in GSH levels in automechanics compared with controls in this study ($P>0.05$).

TAC gives an assessment of the overall antioxidant status (Krajcir *et al.*, 2008). TAC was significantly lower in auto mechanics than controls ($P<0.05$). This indicates increased overall oxidative stress status in auto mechanics arising from exposure to hazardous chemicals. Additionally, TAC in association with GSH and OSI were inversely related while TPP was positively related with years at occupational exposure in automechanics but not in controls ($P<0.05$). There is evidence that exposure to chemicals at work place leads to generation of free radicals which if unaccompanied by available antioxidant leads to oxidative stress. Excess free radical formation and deficit in antioxidant bioavailability in occupationally exposed individuals may serve as an early biochemical indicator of a pathophysiologic state (Anetor *et al.*, 2009). Esposito (2006) reported that obesity may induce systemic oxidative stress. Increase in obesity-associated OS is probably due to the presence of excessive adipose tissue (Fonseca *et al.*, 2007).

The finding of significantly elevated testosterone levels in automechanics compared with controls ($P<0.05$) was surprising although the levels in both groups were within the normal reference intervals. This finding may be due to increased awareness of and actual increased intake of vegetable and fruit as reflected by adequate non enzymatic antioxidants in the normal weight automechanics in this study. Although our observations are contrary to the finding of Adekola *et al.* (2016), who showed significant hypogonadism in automechanics exposed to mixed chemicals, they hypothesized that testosterone may act as an antioxidant in overwhelming oxidative stress conditions. The contribution of obesity to our observations in this study is limited because both automechanics and controls had normal BMI and similar anthropometry. Fabian *et al.* (2016) had shown associations increased adiposity and reduction in testosterone levels. Most auto mechanics in this study had low level education and benefit from continued education on the intake of fruit and vegetables as well as the use of protective clothing and other safety measures to reduce the level of exposure to mixed chemicals.

Oxidative stress due to exposure to mixed chemicals has been extensively studied in recent years and may lead to hypotestosteronaemia. Auto mechanics are occupationally exposed to hazardous mixed chemicals which generate free radicals thereby causing oxidative stress. Oxidative stress was observed in normal weight auto mechanics as shown in their reduced antioxidant status. However, lifestyle changes through adequate education on safety strategies and high

intake of fruit and vegetables by automechanics might reduce exposure to chemical toxicity and provide adequate antioxidants to ameliorate the observed oxidative stress.

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