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Determination of Lead and some Parameters of Oxidative Stress in Exhaust Fume in Relation to Age in Commercial Tricyclists in Kano Municipal

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ABSTRACT

Implication of particulate participation of exhaust fumes in exacerbating cellular damage has been researched. The purpose of this research is to determine the particulate inhalation of exhaust fume, specifically, Lead with some biomarkers of oxidative stress, among different ages of commercial tricyclists. This was investigated in 120 apparently healthy non smokers' commercial tricyclists and non-drivers in Kano Metropolis, achieved by determining serum Lead level, Plasma Malondialdehyde level, Catalase activity, Vitamin C and Vitamin E levels in the subjects. The subjects were divided into two groups; commercial tricyclists (N = 70-Test) and control group (N = 50-non-drivers). The level of Plasma malondialdehyde was significantly higher ($p<0.05$) in test group (12.92±4.89 μM) compared with control group (5.88±4.91 μM). Similarly, the level of serum vitamin E is significantly higher ($p<0.05$) in test group (32.05±10.66 μg/L) compared with control group (21.42±8.55 μg/L). Serum vitamin C test group (2.02±0.99 mg/dL) shows a significance increase ($p<0.05$) over control group (1.35±0.84 mg/dL). The blood lead level of test group is significantly higher ($p<0.05$) (16.36±8.53 μg/dL) compared with control group (9.44±4.86 μg/dL). There was no statistical significant difference between the test group compared to control group in catalase. Higher plasma malondialdehyde in test group was suggestive of higher oxidative stress in the subjects. As a result of the raised blood lead levels, MDA levels tends to be raised with increase in age, and accumulative in adults than in children as age progresses. Thus, the significance of this study reveals cautiousness to occupational and accidental exposures to exhaust fumes.

Keywords: Exhaust fumes; Lead; oxidative stress; Reactive Oxygen Species; antioxidants

INTRODUCTION

Exhaust emissions from mobile sources, stationary area sources (oil & gas production and industrial), and stationary point sources including industrial, electric utilities (electric generators), commercial and institutional sources, [1] emit 60 percent of nitrogen oxides (NO), 17

percent of hydrocarbons, and close to 90 percent of total particulate emissions. [1] Exhaust fume is a complex mixture of gases and particulate matter (PM). Components include; carbon monoxide, carbon dioxide, sulphur dioxide, lead, nitrogen oxides, aldehydes including benzene and formaldehyde, hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and soot (carbon). The exhaust contains 38 components that are hazardous pollutants and is listed as a probable carcinogen by the NIOSH (National Institute for Occupational Safety & Health), [2] and IARC (International Agency for Research on Cancer). [3]

Lead is a toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. Human exposure to lead is estimated to account for 143 000 deaths every year and 0.6% of the global burden of disease.[4] Recent epidemiological studies have reported that low level lead exposure has a graded association with several disease outcomes such as hypertension, peripheral artery disease, kidney disease, neurodegenerative disease, and cognitive impairment. [5, 6] Lead is a cumulative toxicant that affects multiple body systems, including the neurological, haematological, gastrointestinal, cardiovascular and renal systems. Lead is related to a broad range of physiological, biochemical, and behavioral dysfunctions. [7] Chronic exposure commonly causes haematological effects, such as anaemia, or neurological disturbances, including headache, irritability, lethargy, convulsions, muscle weakness, ataxia, tremors and paralysis. Acute exposures may cause gastrointestinal disturbances (anorexia, nausea, and vomiting, abdominal pain), hepatic and renal damage, hypertension and neurological effects (malaise, drowsiness, and encephalopathy) that may lead to convulsions and death. Children are particularly vulnerable to the neurotoxic effects of lead, and even low levels of exposure can cause serious and, in some cases, irreversible neurological damage. Childhood lead exposure is estimated to contribute to about 600 000 new cases of children with intellectual disabilities every year. [8]

The primary cause of lead's toxicity is its interference with a variety of enzymes because it binds to sulfhydryl groups found on many enzymes. Part of lead's toxicity results from its ability to mimic other metals such as calcium, iron, and zinc that take part in biological processes, which act as cofactors in many enzymatic reactions, displacing them at the enzymes on which they act. Lead is able to bind to and interact with many of the same enzymes as these metals but, due to its differing chemistry, does not properly function as a cofactor, thus interfering with the enzyme's ability to catalyze its normal reaction or reactions. [9] Several epidemiological studies among workers with high occupational exposure to lead have reported associations between lead exposure and oxidative stress markers. [10, 11] Lead potentially induces oxidative stress and evidence is accumulating to support the role of oxidative stress in the pathophysiology of lead toxicity. [12, 13]

Oxidative stress appears to be a possible mode of the molecular mechanism of lead toxicity. Oxidative stress occurs with generation of free radicals. Free radicals are substances with one or more unpaired electrons, which tend to overpower the capacity of antioxidant defense mechanisms. Oxidative stress (as formulated in Harman's free radical theory of aging) is also thought to contribute to the aging process. [14] The mechanism of antioxidant defense is a Pathway that provides protection against harmful effect of free radicals. [15] The mechanism of lead-induced oxidative stress involves an imbalance between generation and removal of ROS (reactive oxygen species) in tissues and cellular components causing damage to membranes, DNA and proteins. [16]

Reactive oxygen species (ROS) have been shown to be involved in the etiology of many inflammatory disorders of the gastrointestinal system. [17] Reactive oxygen species play important roles in cell signaling, a process termed reduction – oxidation signaling. Thus, to maintain proper cellular homeostasis, a balance must be struck between reactive

oxygen production and consumption. [18] Production of reactive oxygen species is a particularly destructive aspect of oxidative stress. Such species include free radicals and peroxides Superoxide anion ($\bullet O_2^-$), Hydrogen peroxide (H_2O_2), Hydroxyl radical ($\bullet OH$), Organic hydroperoxide (ROOH), Peroxynitrite ($ONOO^-$), Alkoxy ($RO\bullet$) and peroxy ($ROO\bullet$). [18]

Antioxidants are substances that inhibit or cause delay in oxidation of a substrate while present in a little amounts. Endogenous antioxidant defenses comprises non enzymatic molecules such as glutathione and enzymatic like superoxide dismutase, catalase and glutathione peroxidase. [19] The nutritional antioxidants such as vitamin C and Vitamin E, together with endogenous antioxidants have direct consequence through different mechanism of reducing oxidative stress markers. [20] Malondialdehyde (MDA), is the end product of lipid peroxidation, and is a good marker of free radical mediated damage and oxidative stress. [21] It has been reported that MDA content increases with increasing heavy metal concentration, as a result of inhaled exhaust fume, thus indicating a concentration-dependent free radical generation. [22] Catalase is an enzyme that converts hydrogen peroxide to water and oxygen, using either an iron or manganese cofactor. It protects cells against oxidative stress. Catalase activity is largely located in most eukaryotic cells, in subcellular organelles known as peroxisomes. [23]

Statement of the Problem

It is an obvious fact that many people are occupationally exposed to high levels of lead through paints, exhausts fumes, battery fumes, leaded gasoline and many other sources. Exposure may also occur through ingestion with food and via inhalation of exhaust fumes, which is mostly unavoidable due to the various activities humans undertake.

Previous studies in dogs and rats have shown that long-term exposure

to high level lead in drinking water predisposes the stomach to ulcer. Symptoms include abdominal pain, confusion, headache, anemia, irritability, and in severe cases seizures, coma, and death. [24] Thus, the interest here is to recognize the adverse health side effects, and to better understand the molecular processes underlying lead toxicity in the etiology of oxidative stress and its degenerate conditions. The largest proportion of human emissions is produced by exhausts of internal combustion engines, especially motor vehicles with petrol engines. [25]

As a result of increased human activity, the ambient concentrations measured especially in urban areas are high, and depend greatly on the density of vehicles, topography and weather conditions. The escalated use of automobile, mostly motor cycle and electric generator, to which Nigerians now depend on as an alternative quick and easier sources, have a great deal of pollution. There is general over reliance on biomass as source of energy in the developing countries. [26]

Limitation of the Research

The scope of this research is focused on the biochemical evaluation of the effects of exhaust fumes on human, associated with oxidative stress caused by per oxidants inorganic substances, specifically lead, present in the exhaust fume, as a result of free radical generated. This research is limited only to assessing some specific bio- indicators of oxidative stress in relation to age in Commercial Tricyclists in Kano Municipal.

Approaches to the Research Problem

This research was aimed at investigating the effects of exhaust fume on apparently healthy non smoker commercial motorcyclists in Kano municipal. This was achieved by the determination of the following;

- Plasma malondialdehyde (MDA) of commercial motorcyclists
- Catalase activity in serum of commercial motorcyclists
- Vitamin C (ascorbic acid) level in serum
- Vitamin E (tocopherol) level in serum and

- Evaluation of blood leads in comparison with current standard.

Therefore, the determination of blood lead and evaluation of their toxic effects in exhaust fumes alongside Plasma Malondialdehyde level, Catalase activity, Vitamin C and E levels, becomes an imperative approach to measure the effects in association to age differences.

MATERIALS AND METHODS

Ethical Approval and Informed Consent

This study was conducted according to the guidelines laid down in the Medical Ethics Manual, 2009. [27] All procedures involving human subjects were approved by the Hospital management board of Kano State ethical committee. Similarly, both written and oral informed consent from the subjects recruited for this research was sought for before blood sample was collected.

Chemicals

All chemicals unless otherwise stated were purchased from Sigma Chemical Company (St Louis, MO) USA.

Subjects

One hundred and twenty (120) apparently healthy male non smokers, aged 18-50 years, among which seventy (70) persons who are occupationally exposed to exhaust fumes were recruited and the remaining fifty (50), Non motorcyclists non smokers were used as control group in Kano metropolis.

Blood Collection

Six milliliters (6ml) of blood sample was collected from each subjects by vein puncture. Two milliliters (2ml) was put in EDTA vacutainer tube, for carboxyhemoglobin determination. And the remaining 4ml was centrifuged at 300rpm for 5 minutes to obtain serum. The samples in bottles were kept in refrigerator until required.

Determination of Lead in Blood

The methods described by Allen, [28] Jon and Van, [29] were used. Serum lead level was assayed by using the absorbance values. The exact concentration of the analyte metal in ppm (μg/ml) was calculated from the calibration plot, using the relationship:

$Y=mx + c$.....1 [28]

Where Y =absorbance reading obtained from the spectrophotometer, m= 0.0949, slope of the plot and c= 0 (intercept of straight line graph) and Xppm(μg/ml) = Y/m .....2 [28]

Measurement of Plasma Malondialdehyde

Plasma malondialdehyde was measured by the method of Ohakawa, [30] with absorbance at 532nm because lipid peroxidation, which generates peroxide intermediates which upon cleavage releases MDA, a product which reacts with thiobarbitutic acid (TBA).

Measurement of Catalase

The method described by Goth, [31] was used by measuring the UV absorbance change of H_2O_2 at 405nm against a blank.

Vitamin C Measurement

Serum vitamin C was assayed by the method of Roe and Kuether, [32] in absorbance at 540nm. It generally takes advantage of its reducing ability (ease of oxidation), to form keto product.

Vitamin E Measurement

Vitamin E analysis was determined according to the method of Fabiane, [33] by measuring the UV absorbance at 536nm. Calculation of enzyme concentration was based on the oxidation of tocopherol by Ferric chloride to form a pink complex of ferrous ions with 4, 7-diphenyl-1, 10-phenanthroline (bathophenanthroline).

Statistical Analysis of Data

Standard error of mean (SEM) from the mean standard deviation were obtained for all data collected during analysis and experiment. Differences between the groups were analyzed statistically, using one – way analysis of variance (ANOVA) and t-test statistical analysis. Instat statistical software was used for the analysis. A confidence level of 95% ($p < 0.05$) was considered significant. Results are mean± standard deviation.

RESULTS

A possible impact of exhaust fumes on humans is shown in Table 1. It is a summary of the parameters evaluated in this study. The result is presented as mean±SD of serum lead level, catalase activity, plasma malondialdehyde (MDA), vitamin C (ascorbic acid), and vitamin E (tocopherol) for both test and control groups. The results show statistical significant difference ($p< 0.05$) in all the parameters estimated between the experimental and control, except for catalase, where there is no statistical significant difference. The level of serum malondialdehyde is significantly higher ($p<0.05$) in test group (12.92±4.89 μM) compared with control group (5.88±4.91 μM). Similarly, the level of serum vitamin E is significantly higher ($p<0.05$) in test group (32.05±10.66 μg/L) as compared with control group (21.42±8.55 μg/L). Serum vitamin C test group (2.02±0.99 mg/dL) shows a significance increase ($p<0.05$) over control group (1.35±0.84 mg/dL). The blood lead level of test group is significantly higher ($p<0.05$) (16.36±8.53 μg/dL) compared with control group (9.44±4.86 μg/dL).

Table 1: Summary of Results Showing Lead and Markers of Oxidative Stress

Group	Lead (μg/dL)	Catalase (Ku/L)	MDA (μM)	Vitamin C (mg/dL)	Vitamin E (μg/L)
Test N = 70	16.36±8.53[a]	9.97±8.97	12.92±4.89[b]	2.02±0.99[c]	32.05±10.66[d]
Control N = 50	9.44±4.86[a]	8.22±7.23	5.88±4.91[a]	1.35±0.84[c]	21.42±8.55[d]

Results are mean ± standard deviation, figures in same column bearing same superscript are statistically significant at P <0.05, between the group. N= number of sample.

The effect of lead as free radical generator on the activities of some biomarkers of oxidative damage as related to the ages of subjects used in this study (Motorcyclists) is shown in Table 2. The results show statistical significant difference (p< 0.05) in all the parameters estimated between the test group and control group, except for catalase, where there is no statistical significant difference for age group 31 - 41.

For 18 – 30 age group, there is significant difference (p<0.005) across the groups A and B. The blood lead level is significantly higher (p<0.05) in test group (16.77±8.02 μg/dL) as compared with the control group (7.28±3.50 μg/dL) for the biomarkers; plasma malondialdehyde is significantly higher (p<0.05) in test group (12.06±4.99 μM) as compared to control group (6.46±5.64 μM), Vitamin C is significantly higher (p<0.05) in test group (1.97±0.83 Mg/dL) as compared to control group (1.55±0.82 Mg/dL) while Vitamin E is significantly higher (p<0.05) in test group (30.84±11.18 μg/L) as compared with control group (21.20±9.15 μg/L). For age group 31 – 41, there is significant difference (p<0.05) across the groups. The blood Lead is significantly higher (p<0.05) in test group (15.70±7.77 μg/dL) as compared with control group (7.93±3.73 μg/dL). Similarly, for the biomarkers; plasma malondialdehyde is significantly higher (p<0.05) in test group (13.28±4.3 μM) as compared with the control group (7.93±3.73 μM), Vitamin C significantly higher (p<0.05) in test group (1.81±0.67 Mg/dL) as compared to control group (1.08±0.63 Mg/dL) while Vitamin E is significantly higher (p<0.05) in test group (30.49±10.66 μg/L) as compared with control group (18.69±9.90 μg/L). For age group 42 – 50, there is significant increase (p<0.05) across the groups. The blood lead level is significantly higher (p<0.05) in test group (23.12±4.87 μg/dL) as compared with control group (8.94±4.80 μg/dL). Similarly, the biomarkers; plasma malondialdehyde

is significantly higher (P<0.05) in test group (12.91±4.90 μM) as compared with control group (4.24±3.91 μM), Vitamin C is significantly higher (p<0.005) in test group (2.07±1.17 Mg/dL) as compared with control group (1.38±1.02 Mg/dL) while Vitamin E is significantly higher (p<0.05) in test group (31.76±11.10 μg/L) as compared with control group (24.15±6.61 Mg/dL). The corresponding raised levels of lead and the markers of oxidative stress were vitamin E happens to be higher.

Table 2: Harmful Effects of Lead in Relation to Age and Occupation in Association with Oxidative Stress Markers

Group	Age Group	Lead (μg/dL)	MDA (μM)	Catalase (Ku/L)	VitaminC (Mg/dL)	VitaminE (μg/L)
Test N=30	18 – 30 Years	16.77±8.02[a]	12.06±4.99[a]	10.47±9.29[a]	1.97±0.83[a]	30.84±11.18[a]
Control N=18		7.28±3.50[a]	6.46±5.64[a]	7.97 ±6.91[a]	1.55±0.82[a]	21.20±9.15[a]
Test N=23	31 – 41 Years	15.70±7.77[b]	13.28±4.31[b]	8.20 ±6.35	1.81±0.67[b]	30.49±10.66[b]
Control N=14		7.93±3.73[b]	6.714±5.14[b]	9.90±7.35	1.08±0.63[b]	18.69±9.90[b]
Test N= 17	42 – 50 Years	23.12±4.87[c]	12.91±4.90[c]	11.24±10.84[c]	2.07±1.17[c]	31.76±11.10[c]
Control N=16		8.94±4.80[c]	4.24±3.91[c]	6.82±7.52[c]	1.38±1.02[c]	24.15±6.61[c]

Results are means ± standard deviation, figures in same column bearing same superscript are statistically significant at P <0.05, between the groups. N= number of sample.

The duration of exposure to exhaust fume in relation to age group and eating habit at work, as covered in this research is shown in Table 3. The age is grouped into three of 18 – 30 years, 31 – 41 years and 42 – 50 year, with respective exposure of 10 hours daily, 8 hours daily and 11 hours daily. Duration of exposure is a function of time in hours the subjects go out to work for each day. The eating habit of the subjects showed that

those within age range 18 – 30 and 31 – 41 eats during working hours and do not cook at home. Age groups 42 – 50 eat mainly at home as most of them are married men. For age group 18 – 30, with exposure duration of 10 hours, the blood lead level, serum malondialdehyde, vitamin E and vitamin C levels of test group are significantly higher ($p<0.05$) than the control group. For age group 31 – 41, with exposure duration of 8 hours, the blood lead level, serum malondialdehyde, vitamin E and vitamin C levels of experimental group are significantly higher ($p<0.05$) compared with the control group. Similarly, for age group 42 – 50, with exposure duration of 11 hours, the blood lead level, serum malondialdehyde, vitamin E and vitamin C levels of experimental group are significantly higher ($p<0.05$) when compared to the control group. Mean while, there is no statistical significant difference between the experimental group compared to control group of catalase for age group 31 – 41 years, for 8 hours duration of exposure. The table reveals a trend of the longer the duration of exposure, the higher the significant increase of the parameters.

Table 3: Effects of Duration of Daily Activities and Dietary Habit Relating to Age on Serum Lead Level and Markers of Oxidative Stress

Group	Age Group	Duration of work (Hours)	Place of Eating	Lead (μg/dL)	Catalase (Ku/L)	MDA (μM)	VitaminC (Mg/dL)	VitaminE (μg/L)
A N=30	18 – 30 Years	10hours Daily	restaurant & any available source	16.77 ± 8.02^{a}	10.47 ± 9.29^{a}	12.06 ± 4.99^{a}	1.97 ± 0.83^{a}	30.84 ± 11.18^{a}
B N=18				7.28 ± 3.50^{a}	7.97 ± 6.91^{a}	6.46 ± 5.64^{a}	1.55 ± 0.82^{a}	21.20 ± 9.15^{a}
A N=23	31 – 41 Years	8hours Daily	restaurants and any available source	15.70 ± 7.77^{b}	8.20 ± 6.35	13.28 ± 4.31^{b}	1.81 ± 0.67^{b}	30.49 ± 10.66^{b}
B N=14				7.93 ± 3.73^{b}	9.90 ± 7.35	6.714 ± 5.14^{b}	1.08 ± 0.63^{b}	18.69 ± 9.90^{b}
A N= 17	42 – 50 Years	11hours Daily	at home before and after work	23.12 ± 4.87^{c}	11.24 ± 10.4^{c}	12.91 ± 4.90^{c}	2.07 ± 1.17^{c}	31.76 ± 11.10^{c}
B N=16				8.94 ± 4.80^{c}	6.82 ± 7.52^{c}	4.24 ± 3.91^{c}	1.38 ± 1.02^{c}	24.15 ± 6.61^{c}

Results are means ± standard deviation, figures in same column bearing same superscript are statistically significant at P <0.05, between the groups, N= number of sample, A= experimental group, B= control group.

DISCUSSION

The implicated harmful effects of lead along the age groups (Table 2), shows that occupational lead poisoning is mostly related to adults. [34] The significant increase in serum lead level for age 41-50 years compared with age group 18-30 years may be indicative of longer period of exposure to the metal in the higher age group. Furthermore, the increase may be as a consequence of higher body mass of the higher age group, thus accumulate more of the metal than the later. The raised level of Malondialdehyde could be an indication of free radical peroxidation. [34] The blood lead level tends to be raised with increase in age, and accumulative in adults than in children as age progresses. [35] Although these adults seem to have elevated blood lead level, but symptoms may not be pronounced. [35]

Many people around the world, are obviously exposed to the risk of lead poisoning, trace elements from different sources and vitamin deficiency owing to multiple reasons, which may include changes in eating habits in Western and developing countries, lower food concentration in micronutrients, due to intensive agricultural techniques, occupation, life style and most time unavailability of foods. [36] Motorcycle, tricycle, motor vehicles and generators exhaust fumes are among the important sources of air pollution in urban areas, towns and cities of the developing countries like Nigeria, whereas in rural areas, these sources may be scarcely found. [37]

The non significant difference between experimental and control group of catalase in this study, could be attributed to the facts that, catalase is the slowest of the evaluated antioxidant enzymes to respond to an

increased level of free radicals in blood, as its activity has been found to be unstable. It was reported that catalase activity increased in an experiment conducted between smokers and non smokers as (control). However, after smoking was stopped, the increased activity of catalase was returned as those found with control subjects. [38] This information supports our findings, as it explains the non significant difference between the test and control subjects in this study and as such, age has no influence on the activity of this enzyme (Table 2), since there is no significant difference between test and control group of the various age and exposure. [38,39]

Malondialdehyde (MDA), is the end product of lipid peroxidation, and is a good marker of free radical mediated damage and oxidative stress. [21] The reaction of MDA and other aldehyde with thiobarbituric acid produces a red colored product called thiobarbituric acid reactive substances (TBARS) and F2-isoprostanes and their metabolites when measured. TBARS are believed to represent production of malondialdehyde, a peroxidation product of polyunsaturated fatty acids. [41] There was a significant difference in malondialdehyde test and control group, as a result of the exhaust fumes inhaled. As a result of exposure to exhaust fumes (Table 2), the various age groups showed raised level of plasma malondialdehyde, as this may be implicated by free radical agents of toxicity. [40] This probably reflects the increase in lipid oxidation due to either increased production of free oxidative radicals or decreased antioxidant defense mechanisms, or both, as a result of residence free radical generating agents in the body. [42, 40]

As a result of inhaled exhaust fume, MDA content increases with increasing heavy metal concentration, thus, indicating a concentration-dependent free radical generation. [22] Lead does not directly induce the peroxidation of lipids. It however, makes the formation of free oxygen radicals easier. The action of lead is probably a result of the impoverishment of cells and reduction of the total pool of –SH groups

bound with proteins. [43] The level of MDA in the tissue is considered a measure of lipid peroxidation status (Table 1). Lipid peroxidation is linked to the production of superoxide (O_2^-). Presence of high amounts of transition metals such as copper or iron would also favor enhanced generation of hydroxyl (OH^-) from superoxide (O_2^-) through the Fenton reaction. Thus, the increased level of MDA suggests that exhaust fumes stimulate free radical generating capacity. [43] The exhaust fumes level, though low as compared to acute exposure (smokers), was able to raise the level of malondialdehyde (MDA), as seen by a corresponding raised level of lead, which showed that lipid peroxidation took place, as a result of the free radicals generated. The Level of the MDA would have been much higher among the test group if the two groups have the same level of vitamin intake. [44] Raised level of lead is associated to increase in age, [45] this claim is confirmed in this research (Table 2) with raised level of blood lead level in adults (42 – 50years).

The significant difference in vitamin E and Vitamin C, as antioxidants in the test compared with control group, may be as a result of higher intake of the vitamins among the tricyclists commercial drivers. The per capita income of Nigeria is $2,722. [46] Hence due to the competing demand of many services for the meager resources, many Nigerians cannot afford to buy food rich in antioxidants. However, Tricyclists have reasonable income which account for their ability to take balance nutritional diet. Increased intake of dietary antioxidants including vitamin E, vitamin C, and β-carotene, are associated with reduced risk of atherosclerotic diseases. [47] Thus, antioxidants seem to prevent the development and progression of arteriosclerosis implicated by free radicals. [48] In 1999, the American Heart Association Science Advisory recommended the consumption of a balanced diet with emphasis on antioxidant-rich fruits, vegetables, and whole grains. [49] Food sources of vitamin E include; nuts and seeds, spinach, pumpkin seeds, bell pepper, pine nuts, asparagus, avocados, tomatoes, vegetable oils, papaya, butter, cereals and peanuts. [50] The significant increase in vitamin E level in the

test group over control may be as a result of the consumption of the expensive but nutritiously rich sources of vitamin E by the former group compare to the latter group, who may not afford these foods. [50] Increase in vitamin E appears to be age related (Table 2), as there is corresponding increase in vitamin E of test group over control group with age. However, this could be attributed to the eating habit and quality of food. [50] Thus, increased value of vitamin E has been reported to have effects on the increase in activity of other antioxidants enzymes. This of course, involves its action as an antioxidant, reducing the level of free radicals and, hence, free radical damage. [51]

The progressive increase level of Vitamin E as age increases (Table 2) could explain why symptoms of lipid peroxidation may not be seen obviously in these adults, despite their occupational exposure to exhaust fume, thus, exerting its protective effects. [52,53] An occupational and environmental history with attention to possible lead exposure as revealed by this study buttress the fact that the subjects are regularly exposed and also there are evidences of accumulation of lead and also potential physiologic problems. [35]

Although, the level of vitamin C in this study is low, but statistically significant higher in Tricyclists compared with control. Low levels of Vitamin C (ascorbic acid) are strongly linked to high lead levels and that Vitamin C can inhibit lead uptake at a cellular level as well as lead's cytotoxicity (cellular toxicity). [54] Vitamin C major role is to neutralize free radicals, since it is in a unique position to scavenge aqueous peroxyl radicals before these destructive substances have a chance to damage the lipids. Vitamin C works along with vitamin E, a fat-soluble antioxidant, and the enzyme glutathione peroxidase in order to stop free radical chain reactions. [55] Thus, the reduced plasma levels of vitamin C in this study appear to reflect levels of oxidative stress. [56] Higher level of Vitamins E and C in the occupational workers despite higher plasma malondialdehyde (MDA) may suggest higher intake

among the subjects due to a better income generated by the occupational activity than the control. [50,57] However, the Centers for Disease Control has set the standard elevated blood lead level for adults to be 25 (μg/dl) of the whole blood. For children however, the number is set much lower at 5 (μg/dl) of blood as of 2012 down from a previous 10 (μg/dl). [59,60] However, the measured blood lead level of the subjects in this study, even though lower than the set standard, does not present a save bench mark. [58,60] It is pertinent to understand that, what could be considered as safe limit to the Western societies could be lethal to the developing countries due to disparity in the quality of life.

CONCLUSIONS

In conclusion, this study reveals harmful effects of exposure to exhaust fumes that could results in oxidative stress in humans because of the presence of lead and other particles as agents of free radicals. This could be seen as depicted by a raised level of oxidative stress parameters in the subjects studied. From the results obtained, the significant differences observed in the serum mean±SD of the various parameters evaluated between the test group and control group, revealed a significant level of free radical and peroxidation, as indicated by the raised level of Plasma Malondialdehyde (MDA) and decrease in Catalase activity couple with increase in the levels of non- enzymatic antioxidants (Vitamin E). The blood lead level measured, even though lower than the reference standard by the Center for Disease and Control, showed revealed levels of concern and also established increased blood lead levels with increase in age. This study also shows the difficulty in controlling the effects of free radical generation from exhaust fumes in human subjects since it was not possible to restrict or monitor their life style and eating habits; therefore, the effects of duration of exposure to fumes tend to be quickly neutralized. This study also shows that lead poisoning is accumulating and that adults tend to have more blood lead as age progresses (Table 2). Thus, there is an obvious implication of oxidative stress in the subjects

as a result of increased blood lead level coupled with increase in level of oxidative stress parameters.

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