



Evaluation of antioxidant vitamins among roadside gasoline dispensers in Gombe, Nigeria

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ABSTRACT

Background: Occupational exposure to toxic chemicals such as gasoline is a major public health concern worldwide. Gasoline is one of the most frequently utilized chemicals whose use is on the increase. When absorbed, the volatile constituents of gasoline generate free radicals leading to oxidative stress. In order to maintaining homeostasis and protect itself against the effects of reactive oxygen species and their derivatives, the body has developed a robust mechanism using substances known as antioxidants. Antioxidants can either be a vitamin like beta-carotene, vitamin C and vitamin E or an enzyme like catalase, superoxide dismutase, glutathione peroxidase and thioredoxin reductase. **Methods:** This is a cohort study to compare Antioxidant vitamins (A, C and E), between 90 roadside dispensers of gasoline and 90 age and sex matched controls. Vitamins A, C and E assays were performed using an Ultra sensitive and specific HPLC. **Results:** The mean age of the exposed and control groups are 29.03 ± 3.7 and 29.24 ± 3.5 years respectively. The mean plasma levels of vitamins A ($54.45 \pm 6.4 \mu\text{g/dl}$), vitamin C ($0.68 \pm 0.13 \mu\text{g/dl}$) and vitamin E ($0.72 \pm 0.10 \mu\text{g/dl}$), of the exposed were significantly ($p < 0.001$) lower than the controls ($1.29 \pm 0.25 \text{mmol/L}$, $69.82 \pm 5.4 \mu\text{g/dl}$, $1.06 \pm 0.13 \mu\text{g/dl}$, respectively). **Conclusion:** Our data has demonstrated lower levels of antioxidant vitamins in roadside dispensers of gasoline compared to the controls. This is an indication that roadside gasoline dispensers are probably at greater risk of developing chronic diseases associated with increase oxidative stress. Antioxidant supplementation may be of benefit to the road side gasoline dispensers.

KEY WORDS: Vitamins, Antioxidants, Occupational Exposure, Benzene, Chemical Exposure, Gasoline, Gombe.

INTRODUCTION

Occupational exposure to toxic chemicals is a major public health concern worldwide, and gasoline is a leading cause of this occupational exposure. It is one of the most frequently utilized chemicals and the frequency is increasing due to increasing industrialization(1-3) The risk is greatest in the developing countries, including Nigeria, where there are limited facilities to reduce over exposure and the toxic effects of the chemicals. Knowledge on the safe handling and transportation of chemicals is also very limited.

Roadside dispensers of gasoline, which are common sights in Nigeria most especially in the Northern parts, often use mouth to create a vacuum pressure to dispense the product through pipes into the receivers. This may lead to high levels of exposure to gasoline vapors leading to production of ROS that may consume antioxidants including antioxidant vitamins. In Nigeria, there is paucity of literature on the antioxidant levels among roadside dispensers of gasoline. Therefore, understanding and having knowledge of the levels of antioxidants among these people will help in generating data to be used in the prevention and timely intervention of chronic gasoline toxicity especially in roadside dispensers of gasoline.

Gasoline is a petrochemical that is commonly used for fueling automobiles and some power generating machines. It is very volatile, with many organic and inorganic constituents. (4) Activation of some volatile constituents of gasoline like benzene leads to continuous production of reactive oxygen

species (ROS) and consumption of antioxidants in the body. The ROS produced may lead to injury and diseases by causing damage to DNA, RNA, and proteins by chemical reactions such as oxidation, nitration, and halogenation leading to genetic modification and alteration in the functions of important lipids, enzymes and other proteins.(5-6) Chronic exposure to gasoline has been found to be associated with oxidative stress and many diseases especially cancers(7-12). In order to maintain homeostasis and protect itself against the effects of reactive oxygen species and their derivatives, the body has developed a robust mechanism using substances known as antioxidants. These antioxidants consume the reactive oxygen species and their plasma levels may decrease in the process. An antioxidant can either be a vitamin like beta-carotene, vitamin C and vitamin E (and their derivatives) or an enzyme like catalase, superoxide dismutase, glutathione peroxidase and thioredoxin reductase(13). It is noteworthy, that the antioxidants can also be classified base on their lipids (vitamins A and E) and water (vitamin C and the enzymes) solubility. The lipid soluble antioxidants (A and E) complement the role of vitamin C and the enzymes which act as antioxidants in the aqueous phase. (13-15) Therefore their effects are synergistic(13). The synergy can also be seen in the ability of ascorbic acid in enhancing the effectiveness of vitamin E. For example, it may regenerate vitamin E from the chromanoxyl radical.(16) It is therefore, not surprising that the toxic effects of gasoline have been shown to be reversed by dietary antioxidants supplementations.(17-18)

We therefore hypothesized that long-term occupational

exposure to gasoline is associated with decreased antioxidant vitamins. The aim of the study therefore was to determine and compare the plasma levels of antioxidant vitamins E, C and A between roadside dispensers of gasoline and controls in Gombe state, Nigeria.

MATERIALS AND METHODS

Methodology

This is a cohort study approved by the joint Ethical Review Committee of the University of Ibadan/University College Hospital, Ibadan, recruiting otherwise healthy known roadside dispensers of gasoline in Gombe state using multi-staged sampling method. Age and sex matched controls were consecutively recruited from the same environment. Sample collection and analysis was in 2012.

Inclusion Criteria

Only apparently healthy, full time roadside dispensers of gasoline that are one year and above in the trade were included in the study.

Exclusion Criteria

People who work in painting, welding, battery, auto mechanics industries(19) and petrol station attendants were excluded from the study. All pregnant women(20-21) and patients who have other acute or chronic illness such as diabetes, chronic renal failure and hypertension were excluded from the study. Cigarette smokers and patients taking multivitamins supplements and antiretroviral treatment were also excluded from the study (22-23)

Anthropometric Measurements

Height: - This was measured to the nearest centimetre against a flat, vertical surface with the subjects standing upright. A sliding headpiece was brought to the vertex of the subject's head and the reading at this level was taken.

Weight: - This was taken with salter bathroom scale placed on a flat surface. The reading was recorded to the nearest 0.5kg. Body mass Index (B.M.I) was then calculated using the formula

$$BMI (kg/m^2) = \frac{\text{weight in (kg)}}{\text{Height in (m}^2\text{)}}$$

Their waist circumferences to the nearest 0.5 cm, Hip circumferences, waist to hip ratio and blood pressure were measured using standard procedures. Random plasma glucose was done using a glucometer.

The questionnaires were administered and participants were asked to fast for sample collection the next morning. This is to hasten the process of sample collection, separation and storage.

Sample Collection And Laboratory Procedures

Ten mls of fasting venous blood was collected from each of the

180 participants into a heparinised plastic tube. Plasma was separated by centrifugation frozen within an hour of collection. Samples were protected from light using foil's paper and were stored at -200C. Samples were transported to Ibadan in ice packs by flight and were stored at arrival at -20°C till the time of analysis. This was to increase stability of thermolabile analytes like vitamin C. In a study vitamin C was found to be stable for up to eight days at -20°C in the dark.(24)

Vitamins Assay

The plasma vitamins A, C and E were assayed using the Waters 616 HPLC machine manufactured by the Waters Corporation USA. Serum samples previously stored at -20°C were used.

Statistical Analysis

The data was analyzed using SPSS version 20.00. Qualitative data were reported using percentages. The mean, standard deviation, skewness and kurtosis were used to measure the normality of distribution of the quantitative variables. The mean (SD) was reported for quantitative data and comparison was made between the cases and controls.

The normally distributed variables were compared between the two groups using student T-test. The level of significance was fixed at the 5% probability level. Pearson correlation coefficient was used to establish correlation between antioxidant vitamins and the duration of exposure among the exposed groups.

RESULTS

Fifty per cent (90) of the respondents are roadside dispensers of gasoline. Among the controls, 10 (11.1%) are teachers, 9(10%) are farmers, 17(18.9%) are students and 20(22.2%) are other occupations. (Table 1) Fulanis made up to 103(57.2%) of the respondents while 46(25.6%) are Hausa. Bolewa, Tera and others make the remaining 30(17.2%). Only 2(1.1%) of the respondents take alcohol occasionally and only 15(8.3%) do some exercise. All the continuous variables are normally distributed and therefore parametric analysis was used.

Table 2 shows equality of means in terms of age and other anthropometric characteristics between the cases and controls. This is to remove their confounding effects on the outcome of the study. The average duration of exposure was 6.4years (±2.4). Table 3.

Table 1. Occupational distribution of the respondents

	Frequency	Percent
GASOLINE SELLER	90	50.0
TRADER	34	18.9
TEACHER	10	5.6
FARMER	9	5.0
STUDENT	17	9.4
OTHERS	20	11.1
Total	180	100.0

Table 2. Equality of means of anthropometric parameters and blood pressure between exposed and controls

	T test	Mean Dif	P	95% CI	
				Lower	Upper
AGE	-0.39	-0.21	0.69	-1.27	0.85
WEIGHT	-1.91	-2.81	0.06	-5.71	0.09
HIGHT	-0.70	-0.01	0.49	-0.02	0.01
WC	-1.21	-1.47	0.22	-3.86	0.92
HC	-0.90	-0.93	0.37	-2.98	1.12
PR	1.09	9.58	0.28	-7.82	26.98
BMI	-1.76	-0.82	0.08	-1.74	0.10
WHR	-0.89	-0.01	0.37	-0.02	0.01
SBP	-0.47	-9.36	0.64	-48.46	29.73
DBP	-1.21	-1.01	0.23	-2.66	0.64

Table 3. Average duration of exposure among the exposed population

	Mean	Std. Deviation
DURATION OF EXPOSURE (YEARS)	6.4	2.4
AVARAGE WORK HOURS PER DAY	7.9	0.71
TOTAL EXPOSURE (HOURS)	18332	6931.3

ANTIOXIDANTS

The mean plasma values of antioxidant vitamins A, E and C, were significantly lower ($P < 0.001$) in the exposed group than the controls. The mean plasma level of vitamin E is significantly lower in the exposed population ($0.72 \pm 0.10 \mu\text{g/dl}$) compared to that of control ($1.01 \pm 0.13 \mu\text{g/dl}$). The mean plasma level of vitamin C in the exposed population was also lower ($0.68 \pm 0.13 \mu\text{g/dl}$) compared to $1.06 \pm 0.13 \mu\text{g/dl}$ of the control population. Vitamin A showed statistically significant ($P < 0.001$) lower levels in the exposed population ($54.45 \pm 6.4 \mu\text{g/dl}$) compared with that of the control group ($69.82 \pm 5.4 \mu\text{g/dl}$). (Table 4) There was negative correlation between the duration of exposure and antioxidant vitamins (E, A and C) ($p > 0.05$) as shown in table 5.

Table 4. Student's t- test for equality of means between exposed and controls

VARIABLE	EXPOSED MEAN (SD)	CONTROLS MEAN (SD)	P-VALUE
Vit A(ug/dl)	54.45(6.4)	69.82(5.4)	.000
Vit C(ug/dl)	0.68(0.13)	1.06(0.13)	.000
Vit E(ug/dl)	0.72(0.10)	1.01(0.13)	.000

Table 5. Pearson correlation of duration of exposure (hours) and the vitamins

	r	p
Vit A	-0.065	.542
Vit C	-0.145	.172
Vit E	-0.180	.089

DISCUSSION

Gasoline contain some volatile constituents, which, when inhaled, will lead to continuous production of reactive oxygen species (ROS) and consumption of antioxidants. Since oxidative stress is an imbalance between the rate of production of ROS and their removal by antioxidants, oxidative stress can be assessed by assessing either the increase of production of ROS and its oxidizing effects on proteins, lipids and nucleic acids or by decreased levels of antioxidants.(25-26) This study therefore, compared the plasma levels of antioxidant vitamins between the roadside dispensers of gasoline and controls and also to determine the effect of the exposure period on the antioxidants.

From our limited search, this may be the first study in this group in Nigeria. Roadside dispensers of gasoline are less protected from petrol fumes than petrol station attendants because they use mouth to create a vacuum pressure to dispense the product through pipes into the receivers instead of pumps. They also, almost always, stay by the road side waiting for their customers which in itself is associated with increased levels of exposure to gasoline and its constituents. This is suggested by the findings of Navasumrit P. et al among thai population. They showed that cloth vendors and grilled-meat vendors who are not selling petrochemicals but hawking by the roadsides were exposed to significantly higher levels of benzene (a constituent of gasoline) 22.61 ppb and 28.19 ppb respectively than the control group (12.95 ppb; $p < 0.05$). (27) This is greater than the environmental exposure levels found in most European and North American countries of less than 15ppb.(28). From the forgoing discussions, there is a possibility of increasing chemical exposure to other unsuspecting road users.

Remarkably, there is significantly lower level of antioxidant vitamins in the roadside gasoline despcncers than the control groups. Since antioxidant vitamins (A, C and E) play a major role in protecting individuals from petrochemical induced oxidative stress by neutralizing free radicals through donating

one of their own electrons,(29) the reduced levels in exposed subjects may indicate increased consumption as a result of increased gasoline induced free radicals. This observation is similar to the findings of Basso et al(30) who reported lower concentrations of Vitamins A and E among gasoline station workers than control subjects. Other studies have also shown significantly elevated levels of ROS, and significantly decreased anti oxidant enzymes in the gasoline exposed workers compared with the controls.(30-31)

Our study have also supported the findings that showed gasoline toxicity is mediated by oxidative stress and that exposure to gasoline can predispose to all diseases mediated by oxidative stress. Since the toxic effects of gasoline were seen to be reversed by dietary antioxidants supplementations (vitamins A and E),(17-18) our study can be used to advocate antioxidant supplementation in people exposed to gasoline. The negative correlation between antioxidant vitamins and the duration of exposure further demonstrated the possibility of gasoline mediated decrease in antioxidant vitamins and possibility of increase oxidative stress in the roadside dispensers of gasoline.

Many foods are rich in anti oxidants. These include various fruits and vegetables.(32-35) A study found effective suppression of reactive oxygen species generation 30 minutes after consumption of orange, melon, grape, peach, plum, apple, and kiwi juices.(36) These foods can be used to prevent or reduce the oxidative stress induced by exposure to petrochemicals.

There is, therefore, a strong need for going further to find out the effect of antioxidant supplementation on the oxidative stress in the non-gasoline station dispensers of gasoline. This is because many studies have shown a decrease in oxidative stress in people with oxidative stress after antioxidants supplementation.(37-39) If found to be useful; antioxidant supplementation can be advocated to reduce the risk of oxidative stress among the people that are exposed to gasoline. This is made easy due to the availability and affordability of many fruits and vegetables that are rich in antioxidants ranging from tomatoes, grapes, orange, melon and tea.(32, 35-36, 40)

CONCLUSION AND SUMMARY

This study has demonstrated a strong relationship between exposure to gasoline and antioxidant vitamins. This study may be the first of its kind among roadside gasoline dispensers. There is, therefore, a strong need to do a cohort study that involves following the exposed individuals to monitor progression of the oxidative stress or their reversal by anti oxidant supplements or the use of face mask. From the study, none of the exposed people use any form of protection, indicating limited knowledge of the risk involved in their occupation. There is, therefore, a great need for public awareness on the risk involved in road side gasoline dispensing and the ways of minimizing it.

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