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## Dysfunctional Liver and other High Metabolic Organs in Asymptomatic Petroleum Depot Workers in Calabar South-South, Nigeria

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### ABSTRACT

The health concerns for petroleum workers warranted this study in asymptomatic exposed (n=64) and control (n=64) groups. The serum activity (IU L<sup>-1</sup>) in the exposed group for alanine amino transferase, ALT (49.25±5.28), aspartate amino transferase, AST (68.23±6.95), alkaline phosphatase, ALP (59.23±7.21) and gamma glutamyl transpeptidase, GGT (36.06±6.99) was comparatively higher (p<0.05) than that in the control (21.56±7.68, 47.89±7.14, 29.53±6.31 and 18.11±4.00, respectively) by 128.43, 42.47, 100.58 and 99.12%. The serum concentration for total bilirubin, TB (22.89±6.78 μmol L<sup>-1</sup>) and conjugated bilirubin, CB (17.65±4.77 μmol L<sup>-1</sup>) in the exposed group was higher (p<0.05) than that in the control group (11.59±2.10 and 7.53±1.71 μmol L<sup>-1</sup>) by 97.50 and 134.40%, respectively whereas the computed TB:CB ratio (1.30±1.62) was lower (p<0.05) than that in the control group (1.54±2.33) by 15.58%. Aside the AST:ALT ratio (1.39±1.26) that was lower (p<0.05) than that in the control (2.22±1.84) by 37.39%, the other observations in the exposed group for computed serum ALT:AST ratio (0.72±2.42), ALT:ALP ratio (0.83±2.33) and CB:TB ratio (0.77±2.72) were higher (p<0.05) than that in the control (0.45±3.45, 0.73±2.04 and 0.65±3.11) by 60.00, 13.70 and 18.46%, respectively. The study suggests dysfunctional liver and other organs, with the attendant health implications in the petroleum depot workers, highlighting the need for health caution and protection.

**Key words:** Aspartate amino transferase, alkaline phosphatase, gamma glutamyl transpeptidase, diagnostic ratios, total bilirubin

### INTRODUCTION

Petroleum products vapours are organic pollutants and volatile hence persist in the environment (Schechter *et al.*, 2006), could bio-accumulate due to the stability of their complexes with lipids (Kampa and Castanas, 2008) and could be readily inhaled. Humans may be exposed to varying degrees of the petroleum products vapours in the course of daily human activities in the home, service stations, petroleum storage depots, refineries and oil spillage sites (Ujowundu *et al.*, 2011). As pollutants or contaminants, the petroleum products vapours adversely affect the natural environment and human health (Kampa and Castanas, 2008).

The severity of petroleum products vapours-induced effect, notably on human health, depends on the chemical nature, concentration and persistence of the pollutant (Tietenberg, 2006). Obviously, the concentration of and degree of exposure to petroleum products vapours would be higher in the tank farms and gantry areas of petroleum storage depots. The petroleum depot

workers in Calabar metropolis, Nigeria do not wear the personal protective kits. This could enhance their exposure to and the attendant adverse effects from the petroleum products vapours, warranting this study, designed with the objectives set to assess the changes in some serum liver function bioindicators and some of their diagnostic ratios, in asymptomatic petroleum depot workers in Calabar metropolis, Nigeria.

Liver function markers viz: serum ALT, AST, ALP and GGT activity, total and conjugated bilirubin concentration and their corresponding diagnostic ratios, including that of AST:ALT, ALT:AST, ALT:ALP, TB:CB and CB:TB, have been used to assess the physiological status in animals (Siddiqi *et al.*, 2007; Egbuonu, 2010; Egbuonu *et al.*, 2010a, b, 2012, 2013; Egbuonu and Ezeanyika, 2013; Hyder *et al.*, 2013). The results of the present study aside contributing to previous knowledge may form a basis for further studies on the subject.

## **MATERIALS AND METHODS**

**Chemicals and equipment:** The various chemicals used for the biochemical analyses were of analytical grade and product of reputable companies. The chemicals and equipment used were provided by the Chief Technologist, Chemical Pathology Laboratory, University of Calabar Teaching Hospital, Calabar Cross River State Nigeria.

**Experimental design:** The study was conducted on asymptomatic male workers in 10 petroleum depots and male University of Calabar students, in Calabar metropolis, Nigeria. The depot workers neither knew their health status nor wore personal protective equipment. The participants were aged between 18 and 35 and must be either a staff of any of the petroleum depots or a student of the said university for 2-4 years. The age range, 18-35 years and duration, 2-4 years criteria were set for this study to fairly match the age and duration of the control. The study made use of a simple random cluster sampling (Lindell and Whitney, 2001) with standard questionnaire (American College of Sports Medicine *et al.*, 2000) for screening the respondents to select relevant subjects for the study. The respondents were informed with assurance of confidentiality and use of resultant information for the purpose of the study. Their oral consent was obtained before the administration of questionnaire and collection of blood sample. Ethical clearance was obtained from the Ethical Committee of Biochemistry Department, Michael Okpara University of Agriculture, Umudike, Nigeria. All biochemical tests were conducted at the Chemical Pathology Laboratory of University of Calabar Teaching Hospital with the assistance of an appointed laboratory technologist.

**Sample size determination:** A total of 210 male petroleum depot workers who met the set criteria (age range, 18-35; depot staff for 2-4 years) served as the exposed study population. Out of the 210 questionnaires administered to the exposed study population, one hundred and 132, representing 62.86%, were returned. A total of 46 of the returned questionnaires were discarded for various reasons, including improper filling. Twenty two respondents declined consent for sample collection. The remaining 64 respondents representing 30.47% of the exposed study population, that gave their consent for sample collection thus served as the exposed group sample size. Based on the exposed group sample size, the control group sample size was set at sixty-four, applying the set criteria (age range, 18-35; student in university for 2-4 years).

**Blood sample collection:** Venous blood was taken from a peripheral vein on any of the arms of each respondent and transferred into a sterile 10 mL sample bottle, allowed to clot and centrifuged at 2000 rpm for 10 min (using Universal 320 Centrifuge, Hettich-Germany) to obtain the serum.

The serum was separated using a Pasteur pipette into a labeled 5 mL sample container and stored in the freezer until used for the determination of each of the respondent's alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transferase/transpeptidase (GGT) and alkaline phosphatase (ALP) activities, bilirubin (direct/conjugated and total) concentration. The serum samples were thawed (brought out from the freezer and allowed to stand at room temperature in order to attain the ambient temperature), before commencing the biochemical determinations.

### **Biochemical principles and procedures**

**Determination of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activity:** The serum activity for AST and ALT was determined by the method of Reitman and Frankel (1957) based on the colorimetric estimation of oxaloacetate or pyruvate, respectively produced through transamination of aspartate or alanine on reacting with 2-4-dinitrophenyl hydrazine (DNPH). The intensity of the resultant brown-colored hydrazone was measured with a colorimeter at 520 nm and the activity (AST or ALT) calculated from the relation:

$$\text{AST or ALT activity (IU L}^{-1}\text{)} = \frac{\text{Test-test blank}}{\text{Standard-standard blank}} \times K$$

where, K is a constant.

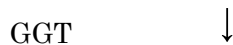
**Determination of serum alkaline phosphatase (ALP) activity:** The serum ALP activity was determined by the method of King and Armstrong (1934). This was based on the colorimetric estimation, at 520 nm, of red colored complex formed following the hydrolysis of phenyl phosphate by alkaline phosphatase to release hydroxybenzene (aside phenol and phosphate) which combines with 4-aminophenazone to form a red complex in a reaction catalyzed by potassium ferricyanide ( $\text{K}_2\text{Fe}(\text{CN})_6$ ). The alkaline phosphatase activity ( $\text{IU L}^{-1}$ ) was calculated from the relation:

$$\frac{\text{Absorbance of test-absorbance of test blank}}{\text{Absorbance of standard-absorbance of standard blank}} \times K$$

where, K is a constant.

**Determination of serum gamma glutamyl transpeptidase (GGT) activity:** The GGT activity in the serum was determined using a reagent kit based on the photometric estimation at 405 nm of 5-amino-2-nitrobenzoate formed as shown in the reaction scheme below:

L-y-glutamyl-3-carboxy-4-nitroanilide+Glycylglycine



L-y-glutamyl glycylglycine+5-amino-2-nitrobenzoate

The GGT activity ( $\text{IU L}^{-1}$ ) was calculated from the relation:

$$\text{GGT activity (IU L}^{-1}\text{)} = \text{Absorbance per min} \times F$$

where, F is a factor equal to 1158.

**Determination of serum bilirubin concentration:** The serum bilirubin concentration (total and conjugated/direct) in  $\mu\text{mol L}^{-1}$  was determined by the method of Powell (1944). This was based on the colorimetric estimation, at 500 nm, of conjugated and unconjugated bilirubin following reaction in aqueous solution with diazotized sulphanilic acid and calculated from the relation:

$$\frac{\text{Absorbance of test-absorbance of test blank}}{\text{Absorbance of standard-absorbance of standard blank}} \times K$$

where, K is a constant.

**Diagnostic ratios:** Diagnostic ratios, including AST:ALT ratio, ALT:AST ratio, ALT:ALP ratio, CB:TB ratio and TB:CB ratio were calculated from the value of corresponding results as obtained in this study.

**Data analysis:** Data was analyzed by simple student t- test using Statistical Package and Service Solutions (SPSS) for windows version 7. Mean differences at  $p < 0.05$  were considered statistically significant. Results were presented as Mean  $\pm$  Standard Deviation (SD).

## RESULTS

As shown in Table 1, the serum activity ( $\text{IU L}^{-1}$ ) in the exposed group for ALT ( $49.25 \pm 5.28$ ), AST ( $68.23 \pm 6.95$ ), ALP ( $59.23 \pm 7.21$ ) and GGT ( $36.06 \pm 6.99$ ) was comparatively higher than that in the control ( $21.56 \pm 7.68$ ,  $47.89 \pm 7.14$ ,  $29.53 \pm 6.31$  and  $18.11 \pm 4.00$ , respectively). This respectively represented a higher and significant ( $p < 0.05$ ) difference by 128.43, 42.47, 100.58 and 99.12% relative to the control.

The serum concentration for total bilirubin ( $22.89 \pm 6.78 \mu\text{mol L}^{-1}$ ) and conjugated/direct bilirubin ( $17.65 \pm 4.77 \mu\text{mol L}^{-1}$ ) in the exposed group was comparatively higher ( $p < 0.05$ ) than that in the control group ( $11.59 \pm 2.10$  and  $7.53 \pm 1.71 \mu\text{mol L}^{-1}$ ) by 97.50 and 134.40%, respectively. However, the computed TB:CB ratio ( $1.30 \pm 1.62$ ) in the exposed group was comparatively lower ( $p < 0.05$ ) than that in the control group ( $1.54 \pm 2.33$ ) by 15.58% (Table 2).

Aside the AST:ALT ratio ( $1.39 \pm 1.26$ ) in the exposed group that was lower ( $p < 0.05$ ) than that in the control ( $2.22 \pm 1.84$ ) by 37.39%, the other observations in the exposed group for serum

Table 1: Serum ALT, AST, ALP and GGT activity in the control and exposed groups

Parameters ( $\text{IU L}^{-1}$ )	Control groups	Exposed groups	Difference relative to control (%)
ALT activity	21.56 $\pm$ 7.68	49.25 $\pm$ 5.28	+128.43*
AST activity	47.89 $\pm$ 7.14	68.23 $\pm$ 6.95	+42.47*
ALP activity	29.53 $\pm$ 6.31	59.23 $\pm$ 7.21	+100.58*
GGT activity	18.11 $\pm$ 4.00	36.06 $\pm$ 6.99	+99.12*

Result: Mean value $\pm$ SD for sample size, n = 64. \*Difference was significant ( $p < 0.05$ ), ALT: Alanine amino transferase, AST: Aspartate amino transferase, ALP: Alkaline phosphatase, GGT: Gamma glutamyl transpeptidase, +: Higher difference, -: Low difference

Table 2: Serum total bilirubin, conjugated/direct bilirubin and TB:CB ratio of the control and exposed groups

Parameters ( $\mu\text{mol L}^{-1}$ )	Control groups	Exposed groups	Difference relative to control (%)
TB concentration	11.59 $\pm$ 2.10	22.89 $\pm$ 6.78	+97.50*
CB concentration	7.53 $\pm$ 1.71	17.65 $\pm$ 4.77	+134.40*
TB:CB ratio	1.54 $\pm$ 2.33	1.30 $\pm$ 1.62	-15.58*

Result: Mean value $\pm$ SD for sample size, n = 64. \*Difference was significant ( $p < 0.05$ ), TB: Total bilirubin concentration, CB: Conjugated/direct bilirubin concentration, +: Higher difference, -: Low difference

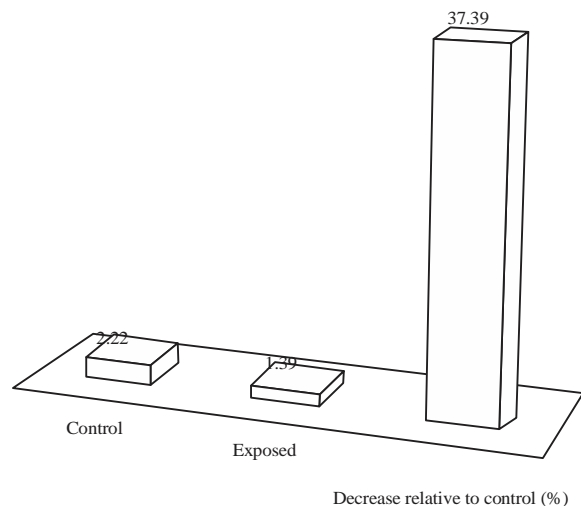


Fig. 1: AST:ALT ratio of the control and exposed groups

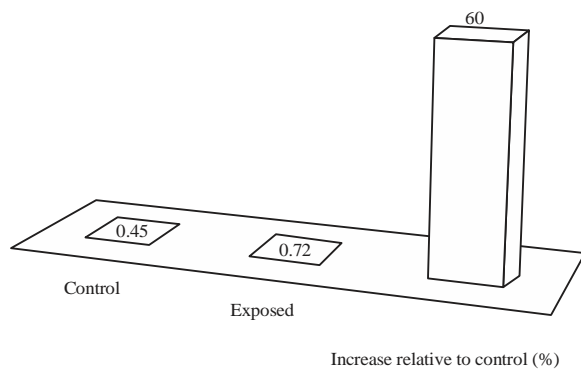


Fig. 2: ALT:AST ratio of the control and exposed groups

ALT:AST ratio ( $0.72 \pm 2.42$ ), ALT:ALP ratio ( $0.83 \pm 2.33$ ) and CB:TB ratio ( $0.77 \pm 2.72$ ), respectively were comparatively higher ( $p < 0.05$ ) than that in the control ( $0.45 \pm 3.45$ ,  $0.73 \pm 2.04$  and  $0.65 \pm 3.11$ ) by 60.00, 13.70 and 18.46% (Fig. 1-4).

## DISCUSSION

Petroleum products vapours are organic, volatile and readily inhalable pollutants that could persist in the environment (Schechter *et al.*, 2006) and bio-accumulate due to the stability of their complexes with lipids (Kampa and Castanas, 2008). Petroleum depot workers in Calabar metropolis do not wear personal protective equipment, which could enhance their exposure to the petroleum products vapours and the possible health risks, warranting this study. The concentration of conjugated bilirubin and that of total bilirubin in the petroleum depot workers were higher ( $p < 0.05$ ) than in the control, suggesting, aside liver dysfunction (Egbonu, 2010), increased destruction of the red blood cells (hemolysis) and impaired platelet production (Patrick-Iwuanyanwu *et al.*, 2013). The significant observation in this study may be ascribed to the high concentration of petroleum products vapours in the depot areas (Bartimaeus and Jacobs, 2003) and perhaps longer period of

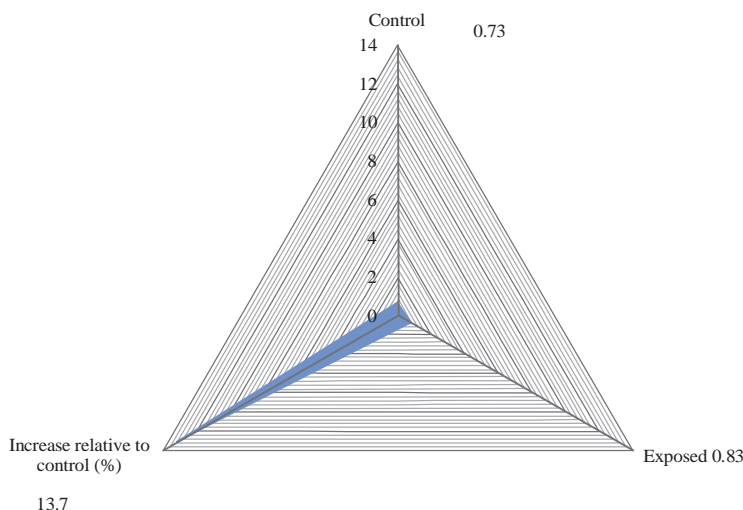


Fig. 3: ALT:ALP ratio of the control and exposed groups

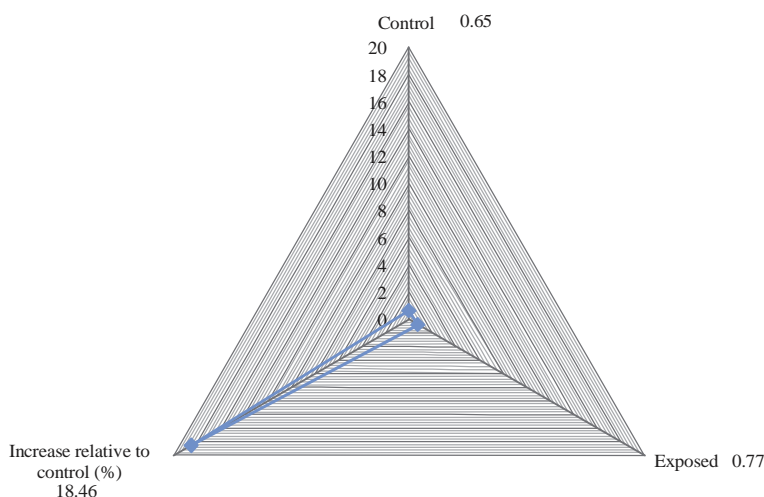


Fig. 4: CB:TB ratio of the control and exposed groups

exposure of the depot workers to the petroleum products vapours. The present result agreed with that of Uboh *et al.* (2005) though in rats exposed to kerosene and petrol vapours.

The higher ( $p < 0.05$ ) alanine aminotransferase (ALT) activity in the exposed group suggests the leakage of the enzyme into systemic blood circulation in apparent response to increased permeability following petroleum products vapours-induced liver damage/injury (McIntyre and Rosalki, 1992). The result is in consonance with that of Nwanjo and Ojiako (2007) in petrol station workers and with that of Patrick-Iwuanyanwu *et al.* (2011) in rats fed on kerosene and petrol contaminated diets. Gamma Glutamyl Transpeptidase (GGT) activity in the exposed group was higher by 99.12% relative to the control group, suggesting liver associated damage in the exposed group (Hyder *et al.*, 2013). The liver damage apparently resulted in response to reactive/free radical species produced during metabolism of the hydrocarbon constituents of the inhaled petroleum products vapours (Bondy *et al.*, 1995).

High serum ALP was associated with liver disease caused by intra or extra hepatic cholestasis and some destruction of hepatic cell membrane (Hyder *et al.*, 2013). The alkaline phosphatase (ALP) activity was higher ( $p < 0.05$ ) in the depot workers which may imply alterations in the synthesis and transport of the enzyme following obstructive liver disease (Halim *et al.*, 1997). This result agrees with that of Nwanjo and Ojiako (2007) in petrol station workers and that of Patrick-Iwuanyanwu *et al.* (2011) but in rats fed on kerosene and petrol contaminated diets. The higher ( $p < 0.05$ ) aspartate amino transferase (AST) activity observed in the exposed group as against the control agrees with the report of Nwanjo and Ojiako (2007) for petrol station workers. Amino transferase (AST) enzyme is not liver specific, thus this could be a pointer to damaged high metabolic organs, aside the liver (Egbonu *et al.*, 2010a).

Generally, when most liver function test results are high, other non hepatic sources need to be considered. In this regard, diagnostic ratios may be beneficial and such uses have been reported (Egbonu *et al.*, 2010a, b; Egbonu and Ezeanyika, 2013; Hyder *et al.*, 2013). The reversal of such ratios, in particular AST:ALT ratio may increase the sensitivity of prediction (Siddiqi *et al.*, 2007). The higher ALT:ALP ratio in the exposed group is suggestive of hepatocellular injury while that of higher CB:TB, reversed as indicated by the TB:CB ratio result (Siddiqi *et al.*, 2007), predicts cholestasis (Yu *et al.*, 2012). The lower AST:ALT ratio in the exposed group relative to the control, though slightly greater than one, may implicate liver among the possible organ damage that resulted to high AST activity in the serum of the exposed group. AST:ALT ratio quite greater than one excluded liver from the possible organ damage related rise in AST activity and vice versa for ratio equal to or less than one (Egbonu *et al.*, 2010a, b). The result and the suggestion thereto were seemingly supported by the reversal ALT:AST ratio of less than one that indicated hepatic cirrhosis (Siddiqi *et al.*, 2007). Generally, these diagnostic ratio results suggest that other organs aside the liver may have been impaired in apparent response to the possibly inhaled petroleum products vapours. The results of this study, especially in concert with that of a similar study (Egbonu *et al.*, 2015) deserve follow up.

## **CONCLUSION**

The study suggests dysfunctional liver and other organs, with the attendant health implications in the petroleum depot workers. The study underscored the need for health caution and protection among the petroleum depot workers.

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