

The Effect of chronic occupational exposure to petroleum products on Haematological and biochemical parameters **on petrol attendants**

## ABSTRACT

**Aim:** The aim of this study was to evaluate the effect of chronic occupational exposure to petroleum products on Hematological indices and liver biochemical profile among petrol station attendants in Enugu, Nigeria

**Subjects and Methods:** Ninety participants in Enugu metropolis were recruited comprising of 30 control group (shopkeepers), thirty (30) petrol attendants exposed to these fumes for < 2 years and thirty petrol attendants exposed for > 2 years. Blood samples were collected from all participants for the determination of full blood count and biochemical parameters such as zinc, copper, lead, liver enzyme markers-aspartate and alanine transaminase.

**Result:** The result revealed a statistically significant decrease ( $P<0.001$ ) in the mean values of total white blood cell count of the group exposed for >2years ( $4.74\pm0.36 \times 10^9$  /L) when compared to the group exposed for <2years ( $5.36\pm0.70 \times 10^9$  /L) and control group ( $5.77\pm0.70 \times 10^9$  /L). Furthermore, there was a significant decrease( $P=0.05$ ) in the mean granulocyte % in the group exposed for >2years ( $43.86\pm10.34$  %) when compared with the control group ( $50.89\pm7.62\%$ ). The result also showed a statistical significant decrease ( $P<0.01$ ) in the mean levels of copper and a statistical significant increase in lead levels( $P<0.01$ ) between test group and control. The liver enzyme marker revealed a statistical significant difference ( $P=0.05$ ) in the mean level of AST.

**Conclusion:** This study demonstrated that petroleum product and its toxic component has a suppressive effect on the total white blood cells, granulocyte, and increases lead content levels and may have an acute hepatotoxic effect with an exponential increase in exposure.

**Keywords:** Petroleum, biochemistry, Haematology, Nigeria, heavy metals

## INTRODUCTION

Nigeria is a developing nation and is the 6<sup>th</sup> largest oil producing country in the world with a production capacity of 2,524,000 barrel per day [1]. Petroleum product such as kerosene, petrol (also known also as gasoline), and diesel are derived from the fractional distillation of petroleum(crude oil). These fractions of petroleum contain aliphatic and aromatic variety of saturated and unsaturated hydrocarbons- benzene, toluene and xylene (BTX) [2]. Petrol is a volatile substance which forms vapour stream, releasing hydrocarbons especially benzene to the environment [3].

Petroleum products are used for many reasons because **of their importance**. Consequently, the need for constant application of petroleum products has led to frequent exposure to humans and environmental pollution [4]. These products are common agents of environmental pollution. Exposure to human is increasingly becoming a public health concern because of its effects on human health [5]. The sources of exposures can be broadly categorized into two: domestic and occupational exposure. However, those exposed due to the nature of their occupation may be the most affected [6].

Occupational exposure is defined as any contact between the human body and a potentially harmful agent or environment in the workplace [7]. Occupational exposed individuals include petroleum refinery workers, tanker drivers, petrol attendant, automobile mechanics and road side gasoline dispensers (also known as Black market traders). Dispensing and storage of fuel are part of the duties of petro attendants. The different route that this product can be absorbed include through inhalation, ingestion, dermal and ocular exposures. Due to the lipophilic nature of petrol, biologic membranes are targeted leading to structural and functional variations upon exposure [8]. Increased metabolism in the liver may substantially trigger the production of free radicals [9]. However, the availability of trace elements and antioxidants may annul effect of the free radicals.

Trace elements are required in the body in minute quantities for proper growth, development, oxidative balance, protection of the membrane and cytosolic components against damage caused by free radicals [10]. Some of these trace element functions as cofactors to antioxidant enzymes during anti-oxidative processes, they include zinc, copper, manganese and Iron [11]. Superoxide dismutase 1 (SOD 1), an important enzyme for dismutation requires copper and zinc as cofactors [12]. In the absence or low levels of these cofactors the human body is left in oxidative stress and this may be detrimental to the individual [13]. Although lead is considered as a trace element by geochemist because of their presence in trace concentrations in various environmental matrices, they are synonymously heavy metals due to their high density and can only be volatilized at high temperature activity [14]. But tetraethyl-lead an organo-derivative of lead found in petroleum and its fractions is volatilized at 0.4mmHg at 25 °c [15].

Since 2000, petroleum product especially petrol has been only commercially available in unleaded forms within the UK and most of Europe [16]. Nevertheless, in Nigeria, leaded petrol is major source of lead in our environment as petrol sold in filling stations may contain lead of 0.6-0.74g [17]. This is because the country as a developing nation is still counted as a poor nation and a lot of distributors and suppliers cut corners to get more money. Since, lead possesses toxic effects which results from long contact and accumulation [18], it may cause oxidative stress by inducing the generation of reactive oxygen species, reducing the antioxidant defense system of cells and interfering with some essential metals needed for antioxidant enzyme activities [14].

Generally, the effect of petroleum constituents have been described as carcinogenic, haematotoxic, neurotoxic and hepatotoxic [19]. Haematotoxic effect is the effect of drugs, chemicals in the environment or workplace [20]. Haematological parameters are the data base of some diagnostic investigation and these parameters are amongst the methods used in the detection of some changes in health status which may not be apparent during physical examination [21].

Currently, haematological abnormalities have been associated with environmental causes. Furthermore, benzene which is a component of petroleum products has been classified as being carcinogenic to humans [22]. Therefore, there is a need to monitor the early possible effects of these products and their toxic components. This is important especially when Nigerian petrol attendants work over the recommended occupational exposure limit of 23mg/m<sup>3</sup> for 10hr work day in 40 hour/ week[23].

**1.1 Aim of the study:** The study sought to evaluate haematological and biochemical changes in petrol attendants exposed to constant petrol products.

## 2. MATERIALS AND METHODS

## **2.1 Subjects**

The study was carried out on a total number of 90 adult human volunteers from Enugu metropolis, aged between 18-30 years. Questionnaires were distributed and duly filled by the participants. A total number of 30 samples were collected by simple random sampling method from each zones- Enugu East, South and North.

## **2.2 Selection of the Study Group**

The test group comprised of subjects presently working as petrol attendants in filling stations. The test group was categorized into 2 groups- subjects working for less than two years and those that had worked for more than two years. Subjects that met this inclusion criterion were shortlisted. The control group comprised of thirty apparently healthy shopkeepers, who freely wanted to participate in this study.

## **2.3. Exclusion Criteria**

The exclusion criteria includes people diagnosed with cardiovascular disorders, those with family history of malignancies, chronic smokers, subjects with chronic renal and respiratory disease, individuals on corticosteroid therapy, radiotherapy or chemotherapy, those filling stations with close proximity to industries and liver damage or disease.

## **2.4 Ethical Consideration**

Ethical approval was duly obtained from the ethical committee of University of Nigeria Teaching Hospital Ituku-Ozalla, Enugu state (UNTH/CSA/329/VOL.5). Written consent of willingness to participate in the study was obtained from all participants. All participants were staff of petrol station who has worked for a particular period of time within the range of the study. All protocol was as declared by international guidelines according to Helsinki declaration.

## **2.5 Sample Collection**

Blood Sample was collected from the volunteers by venipuncture using sterile 10ml needle and syringe. 7mls of venous blood was taken from peripheral vein on the arm of each subject and 4mls immediately transferred into a sterile labeled plain vials while 3mls was transferred into well labeled potassium EDTA anticoagulant vials.

The blood in the plain vial was allowed to clot and retract. It was centrifuge at 18000g, serum extracted, separated into two separate plain vials, one stored in a cold chain chamber for not more than 2 days for trace element measurement and the other for liver enzyme assay.

## **2.6 Laboratory Analysis**

Analysis of haematological parameters-full blood count was done using a haematological auto analyzer Abacus 380 based on the principle of Impedance as described by Dacie and Lewis[24] while analysis of the levels of Lead, copper and zinc was done by the flame atomic absorption spectrophotometry-Air/Acetylene method as described by Baker *et al.*,[25]. The Buck scientific spectrophotometer 210VGP designed to measure metals in solution. The quantitative determination of AST and ALT in the serum was determined using the REFLOTRON PLUS analyzer based on the principle of reflectance photometry.

## **2.7 STATISTICAL ANALYSIS**

All data generated was analyzed using Graph pad prism version 6. Data was presented as mean and standard deviation. Analysis of variance with Tukey post hoc comparison was used to compare between years of duration of occupational exposure. Hypothesis test were performed using two tailed test. P values were considered significant and highly significant when p is less than 0.05 and 0.001 respectively.

### 3. RESULTS

The result of the study revealed a significant decrease ( $P \leq 0.001$ ) in the mean TWBC in the group exposed to these fumes for  $>2$  years ( $4.74 \pm 0.36 \times 10^9/L$ ) when compared with those exposed for  $<2$  years ( $5.36 \pm 0.70 \times 10^9/L$ ) and with the control ( $5.77 \pm 0.70 \times 10^9/L$ ) table 1.

**Table 1 Comparison of haematological parameters in petrol attendants (based on number of years of exposure  $<2$  years,  $>2$  years and control (shopkeepers)**

Haematological parameters	Control Shopkeepers (n-30)	Group Exposed to fumes for $<2$ years (n-30)	Group Exposed to fumes for $>2$ years (n-30)
WBC( $\times 10^9/L$ )	5.77 $\pm$ 0.70	5.36 $\pm$ 0.70	4.74 $\pm$ 0.36**↓
GRANULOCYTE (%)	50.89 $\pm$ 7.62	47.22 $\pm$ 9.39	43.86 $\pm$ 10.34*↓
LYMPHOCYTE (%)	40.48 $\pm$ 7.89	42.66 $\pm$ 12.16	47.3 $\pm$ 10.00
RBC( $\times 10^{12}/L$ )	5.70 $\pm$ 0.87	5.71 $\pm$ 0.79	5.75 $\pm$ 0.63
HB(g/dl)	13.80 $\pm$ 1.35	13.62 $\pm$ 2.05	16.82 $\pm$ 18.80
PCV (%)	41.58 $\pm$ 4.32	42.02 $\pm$ 6.75	41.31 $\pm$ 5.46
MCV (FL)	74.4 $\pm$ 5.52	74.57 $\pm$ 6.64	74.57 $\pm$ 3.81
MCH (pg.)	24.15 $\pm$ 2.15	24.42 $\pm$ 2.71	24.50 $\pm$ 1.39
MCHC (g/dl)	33.02 $\pm$ 0.85	32.83 $\pm$ 0.64	32.62 $\pm$ 0.61
PLATELET ( $\times 10^9/L$ )	219.50 $\pm$ 54.18	256.30 $\pm$ 74.5	228.17 $\pm$ 66.57

↓- Significant decrease

\*\* $P < 0.001$  when compared with group exposed for  $<2$  years and control group.

\* $P < 0.05$  when compared with the control group.

Our study detected a decrease ( $P=0.05$ ) in the mean granulocyte % in the group exposed for  $>2$  years (43.86 $\pm$ 10.34 %) when compared with the control group (50.89 $\pm$ 7.62 %)

Table 2 shows the levels of trace metals- copper, zinc and Lead. The result depicts a statistical significant ( $P<0.01$ ) decrease in the mean copper level in the group exposed to these fumes for  $>2$  years ( $0.72\pm0.48$  mg/l) when compared with the group exposed for  $<2$  years ( $1.07\pm0.59$  mg/l).

Furthermore, there was a significant ( $P<0.01$ ) increase in the mean lead level in the group exposed for  $<2$  years ( $0.65\pm0.43$  mg/l) and the group exposed for  $>2$  years ( $0.75\pm0.79$  mg/l) when compared with control group ( $0.20\pm0.20$  mg/l)

**Table 2: Comparison of trace metals levels in petrol attendants (based on number of years of exposure  $<2$ years,  $>2$ years) and control (shopkeepers)**

Trace metals	Control Shopkeepers (n-20)	Group Exposed to fumes for $< 2$ years (n-30)	Group Exposed to fumes for $> 2$ years (n-30)
Copper(mg/l)	$1.07\pm0.59$	$1.40\pm1.09$	$0.72\pm0.48^{**}\downarrow$
Zinc(mg/l)	$0.49\pm0.58$	$0.25\pm0.51$	$0.25\pm0.29$
Lead(mg/l)	$0.20\pm0.20$	$0.65\pm0.43^{**}\uparrow$	$0.75\pm0.79^{**}\uparrow$

$\downarrow$ - Significant decrease.

$**P<0.01$  when compared with  $<2$ years for copper.  $\uparrow$ - Significant increase

$**P<0.01$  when compared with the control for lead.

The mean levels of aspartate transaminase (AST) and alanine transaminase (ALT) of all participants were evaluated. The result revealed a significant ( $P=0.05$ ) increase in AST in the group exposed to these fumes for  $>2$  years ( $28.48\pm5.84$   $\mu$ /l) when compared with the control ( $23.38\pm2.54$   $\mu$ /l).

**Table 3: Comparison of AST and ALT levels in petrol attendants (based on the number of years of exposure  $<2$ years,  $>2$ years and control (shopkeepers)**

Liver enzymes	Control	Group Exposed to fumes for $< 2$ years	Group Exposed to fumes for $> 2$ years
Aspartate Transaminase( $\mu$ /l)	$23.38\pm2.54$	$26.82\pm1.50$	$28.48\pm5.84^{*}\uparrow$
Alanine Transaminase( $\mu$ /l)	$14.64\pm3.26$	$17.86\pm1.43$	$19.19\pm7.21$

$\uparrow$ - significant increase

$*P<0.05$  when is compared with control group

#### 4.DISCUSSION

Petroleum products are made up of various toxic components which pose a threat to human health, and this threat may increase exponentially with increased exposure to these products. According to Chilcott and Chapd 2007[16] the volatilized fumes of petroleum products contains aliphatic and aromatic compounds. Assessment of haematological parameters, liver enzymes, trace elements, and lead may provide valuable information about the effects of petroleum product on the blood cells and liver enzyme markers.

The result of this study demonstrated a statistical significant decrease in the total white blood cells count and granulocyte percentage. A concomitant decrease was shown as duration of exposure was increased. This may be attributed to the exposure and inhalation to the hydrocarbon (benzene) and other toxic component found in petroleum products that may cause bone marrow suppression or damage to the white cells.

After inhalation of petroleum product fumes through chronic exposure, lower concentrations of saturated hydrocarbons were detected in human and animal blood and can result in membrane function and structure distortion[26]. Chronic exposure to benzene which can be found in petroleum products can reduce the production of white blood cells from bone marrow and cause proliferation of B cell and T cell (WHO 2010). This is in agreement with other work done [27], where he demonstrated a suppressive effect on the Colony Forming Unit-Granulocyte Myeloid progenitor by benzene. The decrease in WBC will result to leukopenia and impair migration of phagocytic cell and lower resistance of virus, bacteria and foreign bodies [28]. These results support the findings of some authors [29] which demonstrated a decrease in white blood cells as the duration of exposure increases. Although in contrary to other findings [4,30] who demonstrated an increase in white blood cell count with an increase in the duration of exposure. The difference in the findings may be that their study was done on animals.

The present study revealed a statistical significant decrease in the mean copper levels among the petrol attendant, while Zinc showed a slight decrease but was not of statistical significance. The decrease in copper levels may suggest an increase activity of the SOD enzyme and utilization trace elements as cofactor in the presence of excess free radicals. Oxidative balance in the human body is maintained by a variety of defence system consisting of antioxidant scavengers (vitamin C and E) and specific enzymes: catalase, superoxide-dismutase (SOD) and glutathione peroxidases that play a role in removal of free radicals (reactive oxygen species-ROS) [31]. Antioxidants are found to be useful in protecting against chemical toxicity [32]. Although zinc and copper are both utilized by the SOD1, studies have shown that zinc which is used for proper folding and stability of the enzymes can still be replaced by other element like cobalt but copper is not replaced [33]. Environmental pollutants and petrol fumes are reported to enhance oxidative stress within cells [34]. Fumes from petroleum products when inhaled maybe metabolised as xenobiotics by undergoing a series of reactions and biotransformation [31]. ROS are produced as unwanted by products during these reactions and biotransformation. SOD1 is an important enzyme for removal of the ROS (dismutation). Copper and zinc are required as cofactors for its function. This finding is similar to other work [9] which also demonstrated a decrease in the trace element which was found to be is proportionally related to the increase in the duration of exposure.

The mean lead levels in this study result revealed a significant increase for both <2 years and > 2 years subject exposed when compared to the control group. This significant difference in the mean levels of lead was further noted as duration of exposure increased. This increase is beyond the permissible level which is 0.05mg/dl [35]. This may be attributed to the volatility of tetra ethyl lead or burning fumes from the cars exhaust, the lack of personal hygiene of the

attendant (eating around the station, carelessness while dispensing) and lack of supervision to work ethics. The source of lead in the petroleum products may be from storage tanks, from anthropogenic sources such as refining process, natural presence of these metals in the source rock from where the crude oil was extracted [3] and Tetraethyl lead a derivative of lead used as an antiknock agent in petrol. Lead ordinarily cannot be volatilized but tetraethyl lead can be volatilized at 0.4mmhg at 25<sup>0</sup>c [15]. Accumulation of lead in the body is may result in cytotoxic effects [29]. This may be another cause of bone marrow suppression. This findings agrees with a previous study [36]. However, this is in contrast to the work of Serekara *et al.* (2013) [37] who reported no statistical difference when compared to control, which he attributed to the exposure of the inhabitant of that state to gases from petroleum refining company located in Port Harcourt.

The liver plays a central role in the metabolism and excretion of xenobiotic which makes it susceptible to adverse effects [38](Singh et al. 2011). The enzymes aspartate transaminase and alanine transaminase are enzymes majorly found mainly in the liver, but also found in blood cell, heart cells, muscles cell [39]. Although these enzymes are not specific (especially AST) they can be used in primary diagnosis of liver diseases. Observations from the results revealed an increase in the levels of AST. Although the ALT was not statistically significant it showed a slight and gradual increase, this may suggest that exposures to environmental toxic compounds may cause an adverse effect on the liver. Increased levels may be due to leakage of the enzymes primarily as a result of cell membrane increased permeability. Also, metabolism of poisonous chemical substances takes place in the liver, which accounts for the organ's susceptibility to metabolic induced hepatotoxicity [20]

Furthermore, results evaluated from the questionnaire included a list of uncommon irregular health experiences by these attendants, some of which included respiratory disturbances, cough and discoloration of the skin, irregularities in their menstrual cycle. A similar report was on the effect of acute toxicity of petrol in different animal species as primarily dermal irritancy, acute dermal irritancy, and pulmonary disturbances [16]. Similarly, the effects of the toxic component of petroleum products especially benzene, nickel were described as irritation, rash, dermatitis, and discoloration [40] (ATSDR 2007).

## **5.CONCLUSION**

Owing to the findings of this study, the effects of constant exposure to petroleum products by petrol attendants may include haematotoxic, oxidative imbalance, chronic lead intoxication and hepatotoxic effect.

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