Comparative Haematology and Urinary Analysis of Passive Inhalers of Petrol Fumes (Petrol Station Attendants) in Benin City, Nigeria

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ABSTRACT

The study investigated the effects of exposure to petrol fumes on haematological parameters and urine composition of petrol station attendants in Benin City, Edo state, Nigeria. 2ml venous blood and 5ml voided urine samples were collected from volunteer petrol station attendants and subjected to haematological and urine composition analysis. Results of haematological analysis indicated significant changes in haemoglobin concentration, packed cell volume, red blood cell and white blood cell counts ($p>0.05$). Urine composition analysis indicated high levels of bilirubin, urobilinogen and nitrites in the voided urine of exposed subjects. Urine analysis also revealed complete absence of ketone, glucose and leucocytes in the urine of all exposure groups. Degree of these changes was observed to depend on exposure duration. Findings from this study indicates that exposure to petrol fumes has haemotoxic implications with high possibility of inducing anaemia evidenced by significant reductions in red blood cell count as well as possible immune depressing effects, indicated by significant reduction in white blood cell count and the presence of nitrites in the voided urine of exposed individuals.

Keywords: Haematology, Petrol fumes, Petrol station attendants, Urine composition, Passive inhalers, Haemotoxic.

1. INTRODUCTION

Petrol is one of the largest volume commercial products in the world and is largely a mixture of normal and branched-chain aliphatic hydrocarbons with boiling points of 39-204^{o}C, and composition that depends on the source of raw material (crude oil), the refining processes, performance specifications, season and other factors (Gabriel *et al.,* 2007). According to WHO (2011), acute poisoning with petrol usually arises from inhalation of vapour as a result of industrial accident or deliberate abuse (sniffing or ingestion) and many of the effects of exposure to petrol include headache, dizziness, nausea, vomiting, confusion, tremor, disorientation, coma and cardiac arrhythmias.

Petrol station attendants (PSAs) are individuals (male or female) responsible for dispensing petroleum products such as Premium Motor Spirit (PMS) at automobile re-fueling stations. In Nigeria, most PSAs dispense fuel into vehicles without using any protective device to minimize their exposure to fumes. Therefore, automobile re-fueling stations are reasonable sources of exposure to petrol vapours among PSAs in Nigeria. This raises serious public health concern since this class of workers is usually not provided with regular medical check-ups to detect potential risk of exposure to the fumes.

The toxicity of gasoline, benzene, toluene and xylene has been investigated in both short and long term exposures. Human studies have often involved acute environmental (accidental and deliberate inhalation) and chronic occupational exposure to gasoline or to a mixture of gasoline components (particularly the aromatic components) while studies using laboratory animals have often focused on subacute and subchronic effects from exposure to gasoline and its major constituents (Uboh *et al.,* 2009). With this in view, this study was designed to investigate the effects of exposure to petrol fumes on haematology and urine composition of apparently healthy but consistent human passive inhalers of the fumes using volunteer petrol station attendants in Benin City, Nigeria.

2. MATERIALS AND METHODS

2.1. Ethical Consideration

Experimental protocols and procedures used in this study conform to the position statement and guidelines regarding toxicological studies by Ethics and research committee of the University of Benin, Benin City, Nigeria. Approval was given by the committee before the commencement of the research work with number AEB/UBREPC/059.

This study was carried out for a period of one week on adult human subjects aged between 18 to 25 years who gave informed consent to the study. Five fuel stations located in Benin City metropolis were used as sites for this study. A total of twenty five subjects took part in this study, consisting of five groups of five individuals each. Grouping was done on the basis of exposure duration in years (0, 1, 2, 3 and 4 years). Group 0 served as the control which is made up of students of the University of Benin who have never worked as fuel station attendants. The PSAs work on the average of 8 hours per day and approximately 50 hours per week. The basis of comparison between the experimental and control group was age and not educational status.

2.2. Collection Of Blood Samples

Venous blood (2ml) was taken from a peripheral vein on the arm of each subject using sterile syringes and immediately transferred into sterile bottles containing potassium EDTA anticoagulant for the haematogical analysis. The blood samples obtained were kept in wet ice prior to haematogical analysis.

2.3. Collection Of Urine Samples

Voided urine samples (5ml) were obtained from willing subjects using sterile urine sample bottles and immediately transferred to the urinalysis laboratory of the University of Benin teaching Hospital for assessment of urine composition.

2.4. Haematological Analysis

The whole blood samples were analyzed using KX-21N automated machine (Sysmex Corporation, Kobe-Japan) following the manufacturer's instructions. Each sample was mixed briefly and placed in contact with the sample probe for aspiration. The unit executed automatic analysis, and the result was displayed on the LCD screen. Haematological parameters analyzed for included: haemoglobin concentration (Hb), packed cell volume (PCV), total red blood cell (RBC) and white cell (WBC) counts, mean corpuscular haemoglobin, (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV).

2.5. Anaysis Of Urine Composition

Analysis of urine composition was done using urinalysis strips (Medi-Test Combi 10 SGL Urine Test Strips ~ Medisave, UK). The strips were dipped in 5ml of voided urine and removed within 60 seconds. Colour developed on the strip was compared with a urinalysis chat indicating semi-quantitative measurements of urine composition.

2.6. Statistical Analysis

This was carried out using student's t-test and ANOVA to compare the mean values of the test groups with the control. $P > 0.05$ was considered to be statistically significant.

3. RESULTS

3.1. HAEMATOLOGICAL ANALYSIS

3.1.1. Haemoglobin Concentration (g/dl)

Mean haemoglobin concentration ranged from 8.35 ± 0.12 g/dl to 12.56 ± 0.01 g/dl in the exposure groups. The mean values were significantly lower than the control value (14.69 ± 0.01) at $p < 0.05$ as shown in table 1.

3.1.2. Packed Cell Volume (%)

Mean values of packed cell volume in the exposure groups ranged from $35.08 \pm 0.35\%$ to 38.69 ± 0.06 % and showed a progressive decrease with increase in exposure duration and vary significantly from the control value (42.63 ± 0.31) at $p < 0.05$, table 1.

3.1.3. Red Blood Cell (RBC) Count (cells/mm^3)

Red blood cell count showed a general decrease with increase in exposure duration. When compared with the control group, mean values of red blood cell count in individuals in all exposure groups were significantly different from the control (table 1).

3.1.4. White Blood Cell (WBC) Count (cells/mm^3)

Mean values of white blood cell count showed a progressive decrease in all exposures groups when compared with the control group and ranged from 3.01 ± 0.14 cells/mm^3 to 3.51 ± 0.01 cells/mm^3 (table 1).

3.1.5. Mean Corpuscular Volume (MCV) (fl)

Mean values of MCV ranged from 90.14 ± 0.60-96.00 ± 1.59fl in exposure groups and showed a general decrease in comparison with the control group (97.17± 0.12fl). MCV in individuals with 3 and 4 years exposure duration were significantly different ($p < 0.05$) from the control group (table 1).

3.1.6. Mean Corpuscular Haemoglobin Concentration (MCHC) (%) and Mean Corpuscular Haemoglobin (MCH) (pg)

Values of mean corpuscular haemoglobin concentration recorded in the exposure groups showed a general increase with increasing exposure duration (table 1) but were lower compared to the control.
With the exception of individuals with four years of exposure duration, mean values of MCH were not significant ($p > 0.05$) when compared with the control value (table 1).

3.2.Urine Composition Analysis

Results of urine composition analysis indicated high presence of nitrites, haemoglobin, bilirubin and urobilinogen in the voided urine of subjects in the four years exposure groups (table 2).

4. DISCUSSION

4.1. HAEMATOLOGICAL ANALYSIS

4.1.1. Haemoglobin Concentration (g/dl)

Mean haemoglobin concentration ranged from 8.35 ± 0.12 g/dl to 12.56 ± 0.01 g/dl in the exposure groups. The mean values were significantly lower than the control value (14.69 ± 0.01) at $p < 0.05$ as shown in table 1. Haemoglobin is the iron-containing oxygen transport metalloprotein in red blood cells. According to Eachempati (2007), this decrease with or without absolute reduction in red blood cells is characteristic of anaemia. This finding agrees with the report that exposure to toxic component of petroleum can alter blood chemistry and induce bone marrow hypoplasia in animals resulting to anaemia (Marieb, 1995).

4.1.2. Packed Cell Volume (%)

Mean values of packed cell volume in the exposure groups ranged from 35.08 ± 0.35% to 38.69 ± 0.06 % and showed a progressive decrease with increase in exposure duration and vary significantly from the control value (42.63 ± 0.31) at $p < 0.05$, table 1. Increase haemolysis from exposure to toxicants and other exogenous agents lowers the number of red blood cells per cubic millimeter of blood (Hillman *et al.,* 2005) and may explain the significant reduction in packed cell volume observed in this study.

4.1.3. Red Blood Cell (RBC) Count (cells/mm^3)

Red blood cell count showed a general decrease with increase in exposure duration. When compared with the control group, mean values of red blood cell count in individuals in all exposure groups were significantly different from the control (table 1). Reductions in the red blood cell count observed may not be unrelated to excessive haemolysis and inhibition of haematopoesis in subjects due to exposure to toxic components of petrol fumes. Toxicant administration has been implicated in haemolytic anaemia characterized by reduced bone marrow red blood cell production (Okoro *et al.,* 2006).

4.1.4. White Blood Cell (WBC) Count (cells/mm^3)

Mean values of white blood cell count showed a progressive decrease in all exposures groups when compared with the control group and ranged from 3.01±0.14 cells/mm^3 to 3.51±0.01 cells/mm^3 (table 1).

White blood cells function primarily in body defense against foreign bodies and this is often achieved through leucocytosis and antibody production (Marieb, 1995 and Okoro *et al.,* 2006). Results of the study indicated that white blood cell count decreased significantly in all exposure groups. This observation points to the possibility that exposure to petrol fume may have a compromising effect on humoral immune responses and phagocytic cell migration necessary for the defense of the body against viruses, bacteria and other exogenous substances. Similar observation, including pancytopaenia and total bone marrow aplasia has been attributed to exposure to benzene (d'Azevedo *et al.,* 1996) and leukocytopaenia due to exposure to xylene (d'Azevedo *et al.,* 1996) both of which are important components of petrol fumes.

4.1.5. Mean Corpuscular Volume (MCV) (fl)

Mean values of MCV ranged from 90.14 ± 0.60-96.00 ± 1.59fl in exposure groups and showed a general decrease in comparison with the control group (97.17± 0.12fl). MCV in individuals with 3 and 4 years exposure duration were significantly different ($p < 0.05$) from the control group (table 1). Mean corpuscular volume measures the red blood cell average as a part of a standard complete blood count and gives an indication of the size of red blood cells. Low MCV recorded in all exposure groups is indicative of microcytic anaemia which may be attributed to the haemotoxic effects of petrol fume exposure.

4.1.6. Mean Corpuscular Haemoglobin Concentration (MCHC) (%) and Mean Corpuscular Haemoglobin (MCH) (pg)

Values of mean corpuscular haemoglobin concentration recorded in the exposure groups showed a general increase with increasing exposure duration (table 1) but were lower compared to the control.

With the exception of individuals with four years of exposure duration, mean values of MCH were not significant ($p > 0.05$) when compared with the control value (table 1). Mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) both indicates the haemoglobin content per red blood cell in a sample of blood and aid in the diagnosis of anaemia (Zuckerman, 2007). These observations further confirm the haemotoxic effect of petrol fume exposures.

4.2. Urine Composition Analysis

Results of urine composition analysis indicated high presence of nitrites in the voided urine of subjects in the four years exposure groups (table 2). Normally the urinary tract and urine are free of bacteria. When bacteria find their way into the urinary tract, they can cause a urinary tract infection (UTI). A positive nitrite test result can indicate a UTI (Gallagher *et al., 1990*). This observation which is indicative of possible bacterial infections in these individuals further confirms the immune depressing effect of petrol fume exposure in these subjects. Hemoglobin was also present in the urine of subjects in the four years exposure group (table 2). Hemoglobin is an oxygen-transporting protein found inside red blood cells (RBCs). Its presence in the urine indicates blood in the urine (known as hematuria). The small number of RBCs normally present in urine usually results in a "negative" test. However, when the number of RBCs increases, they are detected as a "positive" test result. Even small increases in the amount of RBCs in urine can be significant. Numerous diseases of the kidney and urinary tract, as well as trauma, medications, smoking, or strenuous exercise can cause hematuria or hemoglobinuria (Grossfeld *et al., 2001*). The Urine analysis also indicated the presence of bilirubin as well as urobilinogen (table 2). Bilirubin is not present in the urine of normal, healthy individuals. Bilirubin is a waste product that is produced by the liver from the hemoglobin of RBCs that are removed from circulation. It becomes a component of bile, a fluid that is secreted into the intestines to aid in food digestion. The presence of bilirubin in urine is an early indicator of liver disease and can occur before clinical symptoms such as jaundice develop. However, Urobilinogen is normally present in urine in low concentrations. It is formed in the intestine from bilirubin, and a portion of it is absorbed back into the bloodstream. Positive test results help detect liver diseases such as hepatitis and cirrhosis and conditions associated with increased RBC destruction known as hemolytic anemia (Harrison, 2005). The presence of bilirubin, a breakdown product of haem, as well as urobilinogen in voided samples points to possible red blood cell breakdown especially in subjects with longer periods of exposure to petrol fumes.

5. Conclusion

The findings of this study provided insight into the effect of petrol fume exposure on some haematological parameters as well as on urine composition of petrol service station attendants in Benin City Metropolis, Nigeria who consistently but passively inhale petrol fumes daily during the process of dispensing fuel to motor vehicles. The findings indicates that exposure to petrol fumes has haemotoxic implications with high possibility of inducing anaemia evidenced by significant reductions in red blood cell count and presence of urobilinogen in urine as well as possible immune depressing effects, indicated by significant reduction in white blood cell count and the presence of nitrites in the voided urine of exposed individuals. Previous research studies carried out were on composite fumes evaporating from kerosene, petrol and diesel and such studies were carried out on experimental animals. Hydrocarbons like benzene, metals like lead and volatile nitrates have all been shown to produce harmful effects on the bone marrow, spleen, and lymph nodes (Marieb, 1995), Petroleum fumes are therefore environmental pollutants that could have serious consequences on haematological parameters in exposed humans.

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List of Abbreviations

1. Haemoglobin (Hb)
2. Packed Cell Volume (PCV)
3. Red Blood Cell (RBC)
4. White Blood Cell (WBC)
5. Mean Corpuscular Haemoglobin (MCH)
6. Mean Corpuscular Volume (MCV)
7. Mean Corpuscular Haemoglobin Concentration (MCHC)
8. ANOVA: Analysis of Variance

TABLES

Table 1: Data Summary of Haematological function indices

Exposure Duration / Parameters (unit)	Control	1 Year	2 Years	3 Years	4 Years
Hb (g/l)	14.69±0.01	12.56±0.01*	11.10±0.12*	10.19±0.06*	8.35±0.12*
PCV (%)	42.53±0.31	38.69±0.06	38.18±0.35	37.28±0.21*	35.08±0.35*
RBC (cells/mm^3)	4.76±0.01	4.61±0.05	3.30±0.05*	3.15±0.05*	3.05±0.03*
WBC (cells/mm^3)	5.45±0.01	3.51±0.01*	3.23±0.07*	3.13±0.66*	3.01±0.14*
MCH (Pg)	31.27±0.04	30.64±0.03	31.13±0.49	31.03±0.49	29.45±0.14*
MCV (fl)	97.17±0.12	95.00±0.09	96.00±1.59	93.13±1.56*	90.14±0.60*
MCHC (%)	35.53±0.01	30.92±0.01	31.23±0.16	32.53±0.13*	33.80±0.03*

(Values represented as mean ± SE), *=significance ($p<0.05$)

Table 2: Semi-quantitative urine composition analysis

Exposure duration	Blood	Bilirubin	Urobilinogen	Ketone	Glucose	Protein	Nitrate	Leucocyte	pH	Specific gravity
0 Years	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	6.0	1.010
1 Year	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	6.0	1.019
2 Years	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	6.0	1.015
3 Years	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	6.0	1.021
4 Years	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	6.0	1.029

(+ve: positive; -ve: negative) Positive signifies presence of a parameter while negative signifies its absence.

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