Impact of occupational exposure to petroleum products on coagulation and white blood cell indices of Petrol station attendants in Nnewi metropolis, Nigeria

Okeke Chizoba O.[1]*, Onyekaomelu Adaolisa G.[1], Umeh Okechukwu E.[2], Ibeh Nancy C.[1], Chukwuma Oluchukwu M. [1]

[1]Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus P.M.B. 5001 Anambra State, Nigeria.
[2]Department of Medical Laboratory Science, Chukwuemeka Odumegwu Ojukwu University, Anambra State.

Abstract

Petroleum products are complex mixture of hydrocarbon compounds that is easily vaporized during handling. Occupational exposure to petroleum product fumes has become a major public health concern especially in developing countries where adequate protective measures are elusive. These fumes are known to be toxic and can cause abnormal alterations in the functioning of many vital organs. This was a comparative cross-sectional study designed to assess the impact of occupational exposure to petroleum products on the Prothrombin time, activated partial thromboplastin time and white blood cell indices of petrol station attendants (PSAs) in Nnewi metropolis. A total of 100 subjects were recruited comprising 50 petrol station attendants and 50 control subjects. Blood sample was collected and used to assay for the prothrombin time (PT), activated partial thromboplastin time (APTT) and white blood cell parameters. Data was analyzed using Statistical Package for Social Sciences (SPSS) version 21. The Prothrombin time was significantly shorter in the Petrol station attendants than that of the control group (P=0.004), while the activated partial thromboplastin time was significantly longer in the petrol station attendants than the control group (P<0.001). The monocytes count was also significantly higher in petrol station attendants than the controls (P=0.009). Also among the petrol station attendants, the males had significantly longer PT, and APTT values compared to their female counterparts (P=0.008 and 0.006 respectively). Occupational exposure to petroleum products has a detectable impact on coagulation parameters (shorter PT and longer APTT values) which makes it needful that the petrol station workers should be provided with appropriate personal protective equipment to minimize exposure.
Key words: Prothrombin time, Activated partial thromboplastin time, Petrol fumes, petrol station attendants, white blood cells.

Introduction

Environmental and occupational exposure to pollutant like petroleum products which is known to contain benzene has toxic effects on different body systems (Azza and Eman, 2020). Occupational exposure to these harmful substances is almost unregulated in most developing countries and in some developed countries there is very little safety

measures observed. Due to the little or no safety measures used when handling petrol fumes by petrol attendants, exposure to the chemicals in the petrol tends to pose the risk of variety of local and systemic diseases.

Petroleum products are mostly hydrocarbons, which is heterogeneous groups of organic substances that are primarily composed of carbon and hydrogen molecules. Some of the most commonly inhaled or ingested hydrocarbons include petrol, lubricating oil, mineral spirit, lighter fluid/naphtha, lamp oil and kerosene (Mowry *et al.*, 2015). Other common sources of hydrocarbons include dry cleaning solutions, paints, spot remover, rubber, cement and solvent. Petroleum product fumes are ubiquitous in our environment and the common sources of contact or exposure includes refineries, oilfields and filling stations (Patrick-Iwaunyanwu *et al.*, 2011). However, the most affected are those who are occupationally and domestically exposed to petroleum products due to the non-use of protective shields.

Petroleum stations are sources of a mixture of hydrocarbon compounds such as benzene and toluene, and hundreds of saturated and unsaturated hydrocarbons. Thus the gases and vapors emitted from petroleum stations results in enormous hazardous pollution (Azza and Eman, 2020). Petrol station attendants work at the fuel stations; they are exposed to a mixture of hydrocarbons in petroleum product fumes through inhalation of vapours during dispensing fuel, ingestion of contaminated edibles and gases emitted from vehicular exhaust (Rekhadevi *et al.*, 2010). The petrol contain volatile organic compounds such as benzene. A number of mechanisms have been proposed to explain benzene-induced toxicity; however, benzene has been established as a carcinogenic contaminant by the International Agency for Research on Cancer. It was explained that toxicity of inhalation of benzene vapour is due to its biotransformation to reactive

oxygen species (Ekpeyong and Asuquo, 2017). Benzene, an important component of petrol, is a widely distributed environmental contaminant and studies have reported haematological disorders associated with the exposure to benzene in the environment. This is because, following inhalation, benzene vapour is rapidly absorbed into the blood and distributed throughout the body. Acute or chronic exposure to petrol fumes which contains benzene and other toxic chemical can lead to an increased risk of aplastic anaemia, leukemia, thrombocytopenia, cardiac arrest or heart attack caused by presences of thrombosis in the coronary artery of the heart, convulsion, body weakness and loss of consciousness. It also has a negative effect on coagulation factors, fibrinolysis, and other blood coagulation test. Generally, the association between exposure to benzene or benzene containing mixtures and certain types of blood disorders has been shown in epidemiological studies in different countries (Carletti and Romano, 2002; Lan *et al.*, 2004; Lan *et al.*, 2005).

According to Saponaro *et al.* (2009) occupational diseases in petrol station workers have been recognized for many years and affect the workers in different ways and the numbers of such work-related diseases are increasing and under-reported in both developing and industrialized countries. Petrol toxicity from inhalation or ingestion can affect many different organs, but the lungs are most commonly affected. A variety of local or systemic problems occurs through this exposure. Skin and eye irritation, narcosis, pneumonitis, atelectasis, abdominal pain, renal failure, cardiac arrhethemias, cardiac arrest, disseminated intravascular coagulation, coma and sudden death may occur after an acute exposure. Uzma *et al.* (2008) found that exposure to benzene from petroleum products vapour caused haematotoxicity among petrol station workers. It is acutely toxic by inhalation,

causing mucous membrane (nose and throat) irritation, neurological and other symptoms due to respiratory failure including headaches, dizziness, nausea, vomiting, confusion, and breathing difficulties and the effects of skin contact include rash, redness, and swelling (Abou-Elwafa *et al.,* 2015). Most of the previous studies focused on the effect of exposure to petroleum products on the haematopoietic system and blood cells and not much attention has been given to determining the effect on the coagulation system. But since fumes from petroleum products have a negative impact on the organs of the body, we hypothesized that there will be measurable impact on the coagulation system as well. This study which was aimed at determining the impact of exposure to petroleum products on coagulation and white blood cell indices, will contribute immensely in evaluating the coagulation hazards associated with exposure to petroleum products in petrol station attendants.

Materials and methods
Study area
The research was carried out among randomly selected petrol station attendants in Nnewi metropolis, Nnewi North Local Government Area of Anambra state. Nnewi is the Second largest city in Anambra State in south-eastern Nigeria. The city spans over 1,076.9 square miles (2,789km2) in Anambra State (Nnewi Alpha Lite, 2015). Nnewi Metropolis comprises four autonomous quarters: Otolo, Uruagu, Umudim, and Nnewichi. Nnewi is renowned for producing great statesmen and leaders of commerce. This Town is also one of the major trading and manufacturing centers of Nigeria and the city is littered with numerous petrol stations that service the large population of the city.

Study design

This is a cross sectional study designed to assess the impact of occupational exposure to petroleum products fumes on the prothrombin time, activated partial thromboplastin time and white blood cell parameters of petrol station attendants (PSAs) in Nnewi metropolis. The study subjects were recruited by consecutive sampling technique.

Questionnaire
A questionnaire was administered to obtain the required information of the participants which included demographic details (age and gender), personal lifestyle (smoking and alcohol), occupational history and duration of exposure (years of work and number of hours per day), and health status (hereditary diseases, drugs taken, and other diseases such as liver diseases, renal diseases, diabetes mellitus, and hypertension).

Sample size

Sample size was calculated using G-power software (version 3.0.10). Power analysis for differences between two independent means was conducted in G-power to determine a sufficient sample size. The calculated sample size of 98 has 80% power to detect a medium effect size at significance level of 0.05.

Study participants
A total of 100 subjects were recruited for the study. They comprised; Fifty (50) petrol station attendants aged 18-30 years and Fifty (50) apparently healthy controls (comprising undergraduate students of College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus) aged 18 – 28 years who had never worked at a petrol station.
Inclusion criteria and exclusion criteria
Apparently healthy Petrol station attendants and control subjects aged 18 to 30 years were included in this study while petrol station attendants and controls that were sick at the time of sample collection, those with known

bleeding disorders, liver diseases and other chronic diseases were excluded from the study.

Ethical consideration and informed consent

Ethical approval was obtained from the Ethics committee of Faculty of Health Sciences and Technology, Nnamdi Azikiwe University and oral informed consent was sought and obtained from all the subjects recruited for the study.

Sample collection

Five (5) ml of venous blood was collected aseptically from each of the subjects. Two and half (2.5) ml of the blood was dispensed in an EDTA container for blood smear preparation (thin film) used for differential white blood cell count and blood film report. The remaining two and half (2.5) ml of blood was dispensed in a container containing 3.8 % sodium citrate in a ratio of 9:1 for the evaluation of APTT and PT. centrifugation was performed at 1000g for 5 minutes and platelet poor plasma separated.

Method for Activated partial thromboplastin time test (Cheesbrough, 2005)

A clean tube was setup and 0.2 ml of well mixed kaolin/platelet substitute was added and incubated in a waterbath at 37˚c for 15 seconds. To the same tube, 0.1ml of the plasma which has been previously warned at 37˚c was added, mixed gently and incubated for exactly two minutes. Then 0.1 ml of 0.025M calcium chloride solution was added to the test tube and a stop watch was started. The tube was tilted to check for clot and time for first clot to be formed was recorded.

Method for Prothrombin time test (Cheesbrough, 2005)

A clean glass tube was set up and 0.2 ml of calcium thromboplastin reagent was added to it and incubated in a water bath at 37˚c for 1-

2 minutes. To the tube, 0.1ml of the plasma was added, a stopwatch was started. The tube was tilted and observed until a clot is formed. The time of clot formation was noted.

The INR was calculated as follows;

$$INR = \frac{(PT\ Patient)^{[ISI]}}{(PT\ Control)}$$

Preparation and staining of thin film for differential white blood cell count

A thin blood film was prepared on a clean grease free slide, and the smear was air dried. The smear was covered completely with Leishman stain. This was allowed to stand for 2 minutes on a staining rack. The stain was diluted with twice its volume of the buffer solution (the slide was almost flooded). This was allowed to stand for 10 minutes. The slide was then washed with the buffer solution, drained and allowed to air dry by keeping it at a slanted position. The differential white cell count was then carried out microscopically using x100 oil immersion objective.

Statistical analysis

The data obtained was analysed using Statistical Package for Social Science (SPSS) version 21. Data was expressed as the Mean ± Standard deviation. The significance of difference in mean values between groups was analysed using student t-test. P<0.05 was considered statistically significant.

Results

There were more female (34) than male (16) petrol station attendants; most (78%) of whom had worked for less than two years. A good number of them (86%) were employed to work on daily basis while the rest either worked weekly or monthly. Use of protective shields was observed only by a minority of the subjects (10%) and none of them admitted

to be a smoker even though 18% of them admitted to be taking alcoholic drinks. About 32% did not experience any symptoms while others had dizziness (32%), coughing and wheezing (2%), disorientation (6%), and other non-stated symptoms (28%) (See Table 1).

The Prothrombin time and INR of the Petrol station attendants was significantly shorter than that of the control group (P=0.004 and 0.003); conversely the APTT of petrol station attendants was significantly longer than the control group (P<0.001). However the PT and APTT of both groups were all within the normal range. The monocytes count was also significantly higher in petrol station attendants than the controls (P=0.009) (See Table 2).

Among the petrol station attendants, the males had significantly longer PT INR, and APTT values compared to their female counterparts (P=0.008, 0.006 and 0.027 respectively) (See Table 3).

There was no significant difference in the coagulation and white blood cell parameters when compared between petrol station attendants that have worked for less than 2 years and those that have worked for 2 years and above (P>0.05) (See Table 4).

The PT and INR of alcohol consuming petrol station attendants were significantly shorter compared to the non-consuming (P=0.034 and 0.030) while the APTT of alcohol consuming PSAs were longer than the non-consumers (P=0.037) (See Table 5)

There was no statistical significant difference in coagulation and white blood cell parameters between petrol attendants that use protective devices and those that do not (P>0.05).

Table 1: Socio-demographic and clinical data of the Petrol station attendants

Variables	Number	Percentage (%)
Gender		
Male	16	32
Female	34	68

Years of Exposure		
< 2 year	39	78
≥ 2 years	11	22
Work Frequency		
Daily	43	86
Weekly	3	6
Monthly	4	8
Use of Face Shield/Protective wears		
No	45	90
Yes	5	10
Smoking		
No	50	100
Yes	0	0
Symptoms experienced		
Non	16	32
Dizziness	16	32
Coughing and wheezing	1	2
Disorientation	3	6
Others	14	28
Alcohol intake		
No	41	82
Yes	9	18

Table 2: Comparison of coagulation and white blood cell parameters between PSAs and control group

Blood parameters	PSAs (n=50)	Controls (n=50)	t- value	p-value
PT (Seconds)	11.43 ± 1.76	12.58 ± 1.70	-2.963	0.004*

INR	0.92 ± 0.08	0.98 ± 0.07	-3.103	0.003*
APTT (Seconds)	46.23 ± 7.28	38.84 ± 4.63	5.418	<0.001*
NEUT (%)	44.32 ± 12.83	45.45 ± 10.93	-0.422	0.674
EOS (%)	3.73 ± 3.46	3.00 ± 2.28	1.000	0.321
MONO (%)	5.65 ± 2.76	4.20 ± 1.92	2.688	0.009*
LYM (%)	46.5 ± 11.41	47.65 ± 10.14	-0.477	0.635

*P < 0.05 is significant, PSAs = Petrol station attendants, PT = Prothrombin time, INR = International normalized ratio, APTT = Activated partial thromboplastin time, NEUT = Neutrophil count, EOS = Eosinophil count, MONO = Monocyte count, LYM = Lymphocyte count

Table 3: Comparison of coagulation and white blood cell parameters between male and female PSAs

Blood parameters	Male PSAs (n= 16)	Female PSAs (n= 34)	t-value	P-value
PT (Seconds)	12.47 ± 1.72	10.94 ± 1.57	2.808	0.008*
INR	0.96 ± 0.07	0.91 ± 0.08	1.939	0.006*
APTT (Seconds)	49.85 ± 8.78	44.49 ± 5.84	2.296	0.027*
NEUT (%)	46.53 ± 12.46	43.25 ± 13.09	0.753	0.456
EOS (%)	2.91 ± 2.21	4.13 ± 3.91	-0.96	0.344
MONO (%)	4.84 ± 2.67	6.03 ± 2.78	-1.284	0.207
LYM (%)	46.07 ± 11.24	46.7 ± 11.69	-0.161	0.873

*P < 0.05 is significant, PSAs = Petrol station attendants, PT = Prothrombin time, INR = International normalized ratio, APTT = Activated partial thromboplastin time, NEUT = Neutrophil count, EOS = Eosinophil count, MONO = Monocyte count, LYM = Lymphocyte count

Table 4: Comparison of coagulation and white blood cell parameters of PSAs based on duration of exposure (years)

Blood parameters	< 2 years (n=39)	≥ 2 years (n=11)	t-value	P-value

PT (Seconds)	11.43 ± 1.81	11.45 ± 1.67	0.008	0.93
INR	0.93 ± 0.09	0.92 ± 0.07	0.186	0.669
APTT (Seconds)	45.43 ± 6.98	49.01 ± 8.03	1.098	0.301
NEUT (%)	45.42 ± 12.49	40.56 ± 14.01	0.006	0.938
EOS (%)	3.72 ± 3.84	3.77 ± 2.28	0.432	0.516
LYM (%)	45.42 ± 10.85	50.22 ± 13.14	0.251	0.619
MONO (%)	5.74 ± 2.91	5.33 ± 2.35	0.258	0.614

*P < 0.05 is significant, PSAs = Petrol station attendants, PT = Prothrombin time, INR = International normalized ratio, APTT = Activated partial thromboplastin time, NEUT = Neutrophil count, EOS = Eosinophil count, MONO = Monocyte count, LYM = Lymphocyte count

Table 5: Comparison of coagulation and white blood cell parameters between alcohol consuming and non-consuming PSAs.

Blood parameters	**Non-alcohol consumers (n=41)**	**Alcohol consumers (n=9)**	**t-value**	***P*-value**
PT (Seconds)	11.62 ± 1.87	10.84 ± 0.96	2.51	0.034*
INR	0.93 ± 0.08	0.89 ± 0.04	2.94	0.030*
APTT (Seconds)	45.15 ± 7.92	46.65 ± 4.51	2.19	0.037*
NEUT (%)	44.97 ± 13.49	42.71 ± 10.45	0.73	0.446
EOS (%)	3.48 ± 3.7	4.71 ± 2.29	0.90	0.340
MONO (%)	5.43 ± 2.41	5.43 ± 2.82	1.24	0.201
LYM (%)	46.25 ± 12.3	47.0 ± 7.72	0.11	0.823

*P < 0.05 is significant, PSAs = Petrol station attendants, PT = Prothrombin time, INR = International normalized ratio, APTT = Activated partial thromboplastin time, NEUT = Neutrophil count, EOS = Eosinophil count, MONO = Monocyte count, LYM = Lymphocyte count

Discussion

Millions of automobiles on Nigerian roads and in many nations run on refined petroleum products such as Premium motor spirit (PMS) or Diesel fuel. Petrol station attendants are occupationally exposed to petroleum product fumes in the course of dispensing to motorist in automobile re-fueling stations. These fumes contains

volatile organic compound (VOC) such as benzene and when inhaled, benzene vapour is rapidly absorbed into the blood and distributed throughout the body.

Among the participants in this study only 5% of the petrol station attendants were using personal protective wears while dispensing petroleum products. The 90% that used no protective wears gives a vivid picture of the risk that vast population of attendants at many fuel stations scattered across the city are exposed to. The need for protective wears as a way of minimizing occupational exposure to petrol fumes cannot be over-emphasized. Thus, this raises a concern to the need for more enlightenment and enforcement of safe occupational practices by health authorities.

In this study, coagulation parameters and white blood cell indices were compared between petrol station attendants and non-petrol station attendants in Nnewi metropolis. The result showed that the mean value of prothrombin time which assesses the extrinsic haemostatic pathway was within the normal range for both groups. But statistical comparison showed that it was significantly shorter in petrol station attendant than non-petrol station attendants. Conversely, the activated partial thromboplastin time (APTT) which assesses the intrinsic haemostatic pathway was also normal in both subjects but statistically it was significantly longer in petrol station attendants than non-petrol station attendants. These findings suggest that individuals that were occupationally exposed to petrol fumes had significant alterations in their coagulation parameters. The mechanism behind this is not clearly understood but may be linked to the effect of the toxic components of petroleum product fumes to the haemostatic mechanism of the body. Benzene which is one of the volatile organic compounds in petrol has been linked to thrombocytopenia due to its damage to the DNA of pluripotential stem cells. Similarly,

hepatotoxicity and nephrotoxicity have been reported following human and animal exposure to unleaded petrol (Periago and Prado, 2005; Adami *et al.*, 2006); since most coagulation factors are produced in the liver the alterations in the coagulation parameters observed in this study may be attributed to the hepatotoxic effect of the exposure to petrol fumes on the subjects. However this assertion is beyond the scope of this research since the liver function of the participants was not assessed. However, a previous study by Ogunneye *et al.* (2014) showed that an increase in the duration of work of the petrol station attendants (which is equivalent to the period of exposure of individual participants to petrol fumes) resulted in an increase in the level of liver function parameters (AST, ALT, ALP and total bilirubin) of subjects.

In this study, the monocytes count was also significantly higher in petrol station attendants compared to the controls. However, there was no significant difference in Neutrophil, Lymphocyte and eosinophil count in both groups. The reason for this finding is not explicit, however the harmful effects of petroleum products inhalation, including benzene and gasoline on blood components have been established by several previous studies. But notably, most of these studies resulted in conflicting findings due to various factors that may limit similar outcomes; such as differences in sample size, duration of exposure to petrol vapors, and applying different safety instructions by the workers due to different work laws and systems that regulate the work at petrol stations in different countries (Lan *et al.*, 2004; Lan *et al.*, 2005; Abou-Elwafa *et al.*, 2015).

There was no significant difference in these parameters based on duration of occupational exposure. Majority (78%) of the petrol station attendants had worked for less than 2 years and thus were exposed only for a short period of time (< 2 years) which may have

limited the effect of these fumes on the parameters. This may also offer explanation to our finding that the significant alterations observed in the coagulation parameters of the petrol station attendants were still within the established reference range for normal individuals. However, this assertion can only be established when the parameters are assessed in individuals that had worked for longer periods of time. It is worthy of note that this was a limitation in this study due to the fact that most of the petrol station attendants were young people mostly females, that worked at fuel stations as a temporary employment while seeking for admission into higher institutions or better paid jobs. Thus the duration of consistent service by these attendants in these fuel stations are usually short, thereby making it challenging to get petrol station attendants that have worked for longer periods of time.

Previous studies have established a potential effect of gender (Moro *et al*., 2016), age (Mamatou *et al.*, 2020), duration of exposure (Udonwa *et al.*, 2009) on some alterations caused by occupational exposure to petroleum product fumes. Our findings shows that when the parameters where compared between male and female petrol station attendants, the males had significantly longer PT and APTT values than their female counterpart. This finding correlates with the work of Abdullah *et al*. (2003) and Aral *et al.* (2011) that suggested that PT levels differs between ages and gender. This is also similar to Ifeanyichukwu *et al*. (2016) who found out that in different group of subjects the PT, APTT, Thrombin time and Platelet count showed gender differences.

The effect of alcohol intake on these parameters of petrol station attendants was also assessed in this study and the results showed a statistically significantly shorter PT in alcohol consuming petrol station attendants when compared to non-alcohol consuming petrol station attendants. This agrees with the findings of Erhabor *et al*. (2013) who stated that the mean PT and APTT values were significantly shortened (decreased) among alcoholics when compared to non alcoholics. Conversely there was a statistically, significantly longer APTT in alcohol consumers compared to non-alcohol consuming petrol station attendants. This disagrees with the study by Erhabor *et al* (2013). The explanation for this observation is not readily well understood but could have a link to the earlier discovery that alcohol consumption causes hypocellularity leading to anaemia, leucopenia, thrombocytopenia and their relative sequelea (Latvala *et al*., 2004).

Blood picture of the petrol station attendants were generally normal with normocytic normochromic red blood cells and the white blood cells counts were within the normal ranges and did not differ from those of the controls except for monocyte count which was significantly higher in petrol station attendants compared to the controls. Though our finding correlates with work done by Ukaejiofo (2006), the absence of observable changes in the peripheral blood cell morphology in this study may be attributed to several factors such as; the period of exposure which may not have been long enough to cause obvious abnormalities in the peripheral blood cells, the concentration of noxious constituents of the petrol may have been at a minimal or low level or a combination of these factors. According to Uzma *et al*. (2008), the extent of petrol-exposure-induced injury to tissues is dependent on quantity, timing and the design of exposure, while Murtala *et al*. (2015) stated that the alterations in haematological indices due to exposure to various components of petrol (such as benzene) are not constant, especially at lower levels.

In this study, about 32% did not experience any symptoms while others had dizziness (32%), coughing and wheezing (2%), disorientation (6%), and other non-stated symptoms (28%). According to a study by Sirdah *et al*. (2013), 1% of petrol station attendants complained of headache and vertigo, morning cough, and shortness of breath, and 2% complained of skin itch, redness, and rash. In their study they made comparison which showed significantly higher rates of nearly all health status items in workers at filling stations of Gaza governorates than control group, with most of them experiencing headache or fatigue in work, eye itches, redness, pain, skin itches, redness and rash, and respiratory complaints, such as fatigue during climbing stairs, repeated sneezing during working hours, shortness of breath in poorly ventilated room, and shortness of breath in the workplace. Their finding was corroborated by that of Kumar *et al*. (2008) which was conducted in India.

Conclusion

This study revealed that occupational exposure to petroleum product fumes has a detectable effect on coagulation parameters (shorter PT and longer APTT values). This re-emphasizes the need for legislation mandating employers of staff who are occupationally exposed to petroleum products to provide appropriate personal protective devices (such as aprons, gloves, facemasks/shield, goggles etc.) for their staffs as well as ensuring strict compliance in the use of this equipment. Also the practice in industrialized nations of using self dispensing pumps can also be adopted in developing nations such as Nigeria as a measure to minimize exposure in petrol stations.

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