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EFFECT OF PETROLEUM PRODUCTS INHALATION ON SOME HAEMATOLOGICAL INDICES OF FUEL ATTENDANTS IN CALABAR METROPOLIS, NIGERIA

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Summary: Haematotoxic implications of exposure to petroleum fumes through inhalation in human subjects were investigated. A total of 400 subjects (200 males and 200 females) aged between 18-30 years participated. Each gender was further categorized into two groups of 100 each for control and test, respectively. The test group was again subdivided into test 1 (T1) and test 2 (T2) in both sexes. T1 subjects were exposed to petroleum fumes for two years and below while T2 subjects were exposed for more than two years. Samples of blood were collected daily and subjected to haematological analysis. The results obtained showed that in males and females, red blood cell counts (10^6 /mm^3) was significantly ($P<0.001$) decreased in T1 (4.4 ± 0.13) and T2 (3.85 ±0.07) compared to control (4.76 ± 0.01). There was a significant decrease ($P<0.01$) in white blood cell counts, haematocrit, haemoglobin concentration, mean corpusclular haemoglobin concentration (MCHC) in both sexes of test groups when compared with control. There was also a significant ($P<0.001$) decrease in mean corpuscular haemoglobin (MCH) and mean corpuscular volume (MCV) in test 2 males compared with control. Most subjects exposed for longer than two years (T2) had significantly ($P<0.001$) lower values of red blood cell count, haemoglobin concentration and haematocrit than those exposed for less than two years. The odds/odds ratio that a subject would become anaemic progressively rose from less than 1 in the control to greater than 1 or infinity on exposure to petroleum fumes. These results indicate that the petroleum fumes cause a reduction in haematological indices which worsens with prolonged exposure.

***Key Words**: Petroleum products ,Haematological indices, Fuel attendants*

Introduction

Fractional distillation of crude petroleum yields different fractions of petroleum of which petrol, kerosene and diesel are constituent parts. These fractions of crude oil contain aliphatic, aromatic and a variety of branded saturated and unsaturated hydrocarbons (Henderson *et al*, 1993; Kato *et al*, 1993; Anderson *et al*, 1995). Occupational exposure to petroleum fumes have been reported to have toxic effects on various organs and systems, and these include respiratory, immune and nervous systems. Organs such as the heart, lungs, skin and kidneys are affected by these toxic effects resulting in various diseases and different forms of genotoxic, mutagenic, immunotoxic, carcinogenic and neurotoxic manifestations(Becker, 1985; Klassen 1990; d'Azevedo *et al*, 1996; Smith *et al*, 1996; Rabble and Wong, 1996; Ross 1996; Rothman *et al*, 1996).

Petroleum products are used for various reasons by human beings at homes, in manufacturing and petrochemical industries. The uses range from fuels for vehicles, cooking and lighting fuels in homes and outside homes, as chemical feedstock for industries as well as for therapeutic reasons (Hockabey *et al*, 1995). The daily use of petroleum products both in and outside petroleum industries may have effects on users, and those who work directly in petroleum industries (those occupationally exposed) are likely to be more affected than their counterparts who do not work in these industries (Smith *et al*, 1993; Carbello *et al*, 1994; Rothman *et al*, 1996).

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), Most often they add up to other environmental and physiological factors already known, to affect

blood parameters and the resultant effect is stress in the animals exposed. These toxic compounds destroy or inhibit the haematopoietic component in the red marrow (Marieb, 1995). Benzene, which is an aromatic hydrocarbon contained in gasoline, is known to induce leukaemia during occupational exposure (Austin *et al*, 1988)

The main objective of this study therefore was to investigate the effect of inhalation of petroleum products on some haematological parameters in humans occupationally exposed namely, Packed cell Volume (PCV), Red blood cell (RBC), Haemoglobin (Hb), Mean Corpuscular Haemoglobin (MCH), Mean corpuscular Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC) and White blood cells (WBC).

Materials and Methods

Subjects: This study was carried out on adult human subjects aged between 18 to 30 years who gave informed consent to the study. Questionnaires were distributed and accurately filled; candidates who met the criteria for participation in this study were admitted into the study. Several fuel stations located in Calabar metropolis were used as sites for this study (test subjects) while shop attendants and students took part as controls. A total of four hundred subjects took part in this study, consisting of two test groups and two control groups of both sexes - two hundred males and two hundred females. The two hundred males were divided into two groups of one hundred each for test and control. A similar division was done for the two hundred females. The test group was further subdivided into test 1 group (people who had worked for 2 years or less) and test 2 group (people who had worked for more than 2 years).

Venous blood (5 ml) was taken from a peripheral vein on the arm of each subject and immediately transferred into sterile potassium EDTA anticoagulant bottles. The blood samples obtained were analysed on daily basis. The Hb estimation was done by the spectrophotometric method using Drabkin's solution while packed cell volume was determined by the use of micro-haematocit method. The total red blood cell and white blood cell were counted using the improved Neubauer counting chamber under an Olympus binocular electric microscope i.e. estimation by haemocytometric method.

Statistical analysis

This was carried out by employing student's t-test to compare the mean values of the test groups with the control. A $P<0.05$ was considered to be statistically significant. Furthermore, the odds and odds ratios for the various RBC indices were calculated to determine the likelihood of subjects becoming anaemic in both control and test groups. Values of odds and odds ratios which were below one were considered unlikely to become anaemic while values above one were more likely to be anaemic, after the method of Bandolier (2006).

Results

The results obtained as shown in Table 1 compares the mean Hb, PCV, RBC, WBC, MCH, MCV and MCHC of the control group with the means of test 1 group and test 2 group for males. Table 2 compares the control group with the test group for females. The Hb, PCV, RBC, WBC, MCH, MCV and MCHC for the control male group were 14.69 $\pm$ 0.01(g/dl); 46.63 $\pm$ 0.31 (%) 4.76 $\pm$ 0.01 (10^6 / mm³), 5.45 $\pm$ 0.013 (10^3 / mm³), 31.27$\pm$ 0.04 (Pg), 99.17$\pm$ 0.12 (fl) and 31.53$\pm$ 0.01(%) respectively.

The values for Test 1 male group for Hb, PCV, RBC, WBC, MCH, MCV and MCHC were 12.36 $\pm$ 0.06(g/dl); 38.84 $\pm$ 0.21(%), 4.07 $\pm$ 0.06 (10^6 / mm³),. 4.40 $\pm$ 0.13 (10^3 / mm³), 30.75 $\pm$ 0.51(Pg), 96.55 $\pm$ 1.56 (fl) and 31.81 $\pm$ 0.08 (%) respectively while the values for Test 2 male group were 11.12 $\pm$ 0.18(gldl); 34.98 $\pm$ 0.57, 3.85 $\pm$ 0.07, 4.49 $\pm$ 0.18, 28.95 $\pm$ 0.26, 91.04 $\pm$0.80 and 31.8 $\pm$ 0.03 respectively. These results show significant differences ($P<0.001$) between the control group and test groups 1 and 2 for HB,PCV,RBC and WBC while MCHC was significant at $p<0.05$. However, MCH and MCV only decreased significantly in test 2 when compared with control , while test 1 was not significant.

The Hb, PCV, RBC, WBC, MCH, MCV and MCHC for the control female group were 13.66 $\pm$ 0.01, 42.81 $\pm$ 0.05, 4.61 $\pm$ 0.01, 4.54 $\pm$ 0.01, 29.74 $\pm$ 0.03, 93.21 $\pm$ 0.09, 31.94 $\pm$ 0.01 respectively. The values of test 1 female group for Hb, PCV, RBC, WBC, MCH, MCV and MCHC were 10.69 $\pm$ 0.06, 32.88 $\pm$ 0.21, 3.65 $\pm$ 0.05, 3.33 $\pm$ 0.66, 29.63 $\pm$ 0.49, 91.16 $\pm$ 1.56 and 32.53 $\pm$ 0.13 while the values for test 2 female group was 9.45 $\pm$ 0.12, 29.18 $\pm$ 0.35, 3.27 $\pm$ 0.05, 3.25 $\pm$ 0.075, 29.18 $\pm$ 0.49, 90.21 $\pm$ 1.59 and 32.39 $\pm$ 0.16 respectively. There were also significant differences ($P<0.001$) between the control group and both test groups for HB, PCV, RBC, WBC and MCHC (Table 2) while MCH and MCV decreased insignificantly. The results show that the values of all these parameters decreased in the test groups for the females ,with the values for test 2 female group being generally less than test 1 female group.

Comparing test 1 with test 2 groups in the males showed statistically significant decreases in test 2 ($p<0.001$) for RBC,HB and PCV while MCH and MCV were significant at $p<0.01$. In females, RBC, HB and PCV were also significantly decreased in test 2 compared to test 1 while WBC, MCH, MCV and MCHC were not significant.

Table 1: Haemotological indices in Male fuel attendants exposed through inhalation to petroleum fumes

Haematological Indices	Non Petrol attendants (Control)	Petrol attendants exposed to fumes < 2yrs (T_1)	Petrol attendants exposed to fumes >2yrs (T_2)
Hb (gldL)	14.69±0.01	12.356± 0.06***	11.12 ± 0.18***
PCV (%)	46.63 ±0.31	38.84±0.21***	34.98±0.57***
RBC($x10^6/mm^3$)	4.76±0.01	4.07±0.06***	3.85±0.07***
WBC($x10^3/mm^3$)	5.45±0.013	4.40±0.13***	4.49±0.18***
MCH	31.27±0.04	30.73± 0.51NS	28.95 ± 0.26***
MCV	99.17 ±0.12	96.55±1.56 NS	91.04±0.80***
MCHC	31.53±0.01	31.81±0.08*	31.8±0.03*

** P<0.05 compared with control, **P<0.01 compared with control, ***P<0.001 compared with control, NS - Not Significant with control, Result are presented as Means ± S.D*

Table 2: Haemotological indices in Female fuel attendants exposed through inhalation to petroleum fumes

Haematological Indices	Non Petrol attendants (Control)	Petrol attendants exposed to fumes < 2 years (T_1)	Petrol attendants exposed to fumes > 2 years (T_2)
Hb (gldL)	13.66±0.01	10.69± 0.06***	9.45 ± 0.12***
PCV (%)	42.81 ±0.05	32.88±0.21***	29.18±0.35***
RBC($10^6/mm^3$)	4.61±0.01	3.65±0.05 ***	3.27±0.05***
WBC(10^3mm^3)	4.54±0.012	3.33±0.66***	3.25±0.075***
MCH (Pg)	29.74±0.03	29.63± 0.49NS	29.18 ± 0.49NS
MCV (fl)	93.21 ±0.09	91.16±1.56 NS	90.21±1.59 NS
MCHC (%)	31.94±0.01	32.53±0.13***	32.39±0.16**

** P<0.05 compared with control, **P<0.01 compared with control, ***P<0.001 compared with control, NS - Not Significant with control, Result are presented as Means ±S.D*

Table 3a: Odds/odds ratio for anaemia in male subjects

RBC	n	Anaemic	Non-anaemic	Odds (Anaemic/Non-anaemic)	Odds ratio
Control	100	37	63	0.59 (59%)	-
Test 1	50	38	12	3.17 (316.67%)	5.37
Test 2	50	48	2	24 (2400%)	40.68
Hb					
Control	100	0	100	0 (0.0%)	
Test 1	50	8	42	0.19 (19%)	0.19
Test 2	50	34	16	2.13 (213%)	2.13
PCV					
Control	100	0	100	0 (0.0%)	
Test 1	50	8	42	0.19 (19%)	0.19
Test 2	50	29	21	1.38 (138%)	1.38

The normal values for RBC, Hb and PCV used for calculating odds were 5.0-5.5 and 4.5-5.0 (10^6/mm^3), 14 -16 and 12-14 (g/dl) and 40-50 and 36-47 (%) in males and females respectively. The odds that a male subject would become anaemic, using RBC as reference, were 0.59, 3.17 and 24.0 for control, T_1 and T_2 respectively .For Hb it was 0.0, 0.19 and 2.13 while PCV was 0.0, 0.19 and 1.38 respectively. The odds ratios for T_1 and T_2 were 5.37 and 40.68 for RBC, 0.19 and 2.13 for Hb and 0.19 and 1.38 for PCV respectively, as shown in Table 3a. Similarly, the odds of a female subject becoming anaemic, with reference to RBC , were 0.10, 6.14 and infinite in control, T_1 and T_2 respectively. For Hb and PCV, controls were 0.15 and 0.18 respectively while T_1 and T_2 were infinite. The odds ratio for T_1 in the control was 6.14 while T_2 was infinite with regards to RBC. For Hb and PCV, the odds ratios for both T_1 and T_2 were all infinite (Table 3b).

Table 3b: Odds/odds ratio for anaemia in female subjects

RBC	n	Anaemic	Non-anaemic	Odds (Anaemic/Non-anaemic)	Odds ratio
Control	100	9	91	0.1 (10%)	-
Test 1	50	43	7	6.14 (614%)	61.4
Test 2	50	50	0	Infinite	Infinite
Hb					
Control	100	13	87	0.15 (15%)	
Test 1	50	50	0	Infinite	Infinite
Test 2	50	50	0	Infinite	Infinite
PCV					
Control	100	15	85	0.18 (18%)	
Test 1	50	50	0	Infinite	Infinite
Test 2	50	50	0	Infinite	Infinite

Discussion

The haematological parameters Hb, PCV, RBC, WBC, MCH, MCV and MCHC provide information on the general state of the blood of the subjects used for this study. This study has demonstrated that exposure to petroleum fumes causes a significant decrease in RBC values, Hb values, PCV, MCHC and WBC of subjects exposed to petroleum fumes for any number of years.The toxic components, especially those in petroleum fumes, have been reported to change blood chemistry and induce anaemia by causing bone marrow hypoplasia in experimental animals(Marieb,1995). This study suggests a similar effect on humans. Toxic constituents of crude oil such as Benzene and Lead are reported to be activated in the bone marrow and the cytotoxic effects observed are mediated through disturbance in DNA function. The resultant bone marrow depression is characterized by inadequate production of red cell and other formed elements (Rabble *et al*, 1996; Synder and Hedli, 1996). This is in line with findings in this study.

White blood cells function primarily in body defense against foreign bodies and this is often achieved through leucocytosis and antibody production (Marieb, 1995; Robbin and Angel, 1976). In this study, the white blood cell count decreased significantly in humans of both sexes exposed to petroleum fumes and the decrease was greater in those exposed for more than two years. Benzene is reported to produce haematological changes ranging from pancytopaenia to total bone marrow aplasia, effected through its myelotoxic action (d'Azevedo *et al*, 1996). Xylene is also reported to cause leukocytopaenia (d'Azevedo *et al*, 1995). The decrease in WBC observed in this study is possibly as a result of pancytopaenia and leukocytopaenia, which may result in impaired migration of phagocytic cells, lower resistance to viruses, bacteria and foreign bodies (Marieb, 1995).The observation in this study is similar to previous findings attributed to toxicity from constituents of crude oil , combined with stress imposed by crude oil hydrocarbons (Ovuru and Ekweozor, 2004; Dede and Kagbo, 2002 and Ndodigha *et al*, 1999).

The odds of a subject becoming anaemic increased progressively from control values to T_1 values and were highest in T_2. The odds were less than one in the control for RBC, Hb and PCV in both sexes, implying that the control subjects were not likely to become anaemic. This is to be expected as they were not exposed to any form of treatment during the experiment.

However, for the subjects exposed to petroleum fumes for two years and below (T_1), the odds rose above control values and sometimes to infinity, implying that these subjects were more likely to become anaemic than not. All the subjects exposed to petroleum fumes for more than two years (T_2) had odds and odds ratios greater than one, implying that they were all likely to become anaemic. Indeed, those that had infinite values were certain to become anaemic and these were mainly among the females, probably due to their normal inherently lower red cell indices. This shows that exposure to petroleum fumes decreases red cell indices in a manner that is duration dependent.

From the results of this study, it is thus concluded that inhalation of petroleum fumes causes depression of total white blood cell count as well as red blood cell count and its dependent haematological indices (PCV, Hb) , MCH and MCV. Petroleum fumes are therefore environmental pollutants that could have serious consequences on haematological parameters in exposed humans.

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