

Research article

BLOOD DYSCRASIA IN WOMEN OF CHILD BEARING AGE EXPOSED TO PMS IN OKADA – ORE LOCAL COMMUNITY EDO STATE, NIGERIA.

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ABSTRACT

Occupational exposure to petroleum has been reported to have toxic effects on various organs and systems. The purpose of this research is to investigate the potential risk associated with exposure of non-pregnant women of child bearing age selling PMS (WSP) in Okada-Ore Road, Edo State, Nigeria using batteries of haematological parameters and level of MetHb as an indirect blood biomarker of exposure to benzene in PMS. 100 women each for both test and control population within the age 18-34 years were recruited into the study. We observed that women exposed to petroleum had a significant higher Met Hb than control group. The red cell indices decreased with increase in years of exposure. Red blood picture of subject within 1-3years of exposure shows that of dimorphic blood picture with microcytic normocytic hypochromic cells as predominant features. Platelet count decreased with level of Subjects above 5years of exposure resulting in mild thrombocytopenia with few blast. The route of exposure to PMS is by mouth suction due to conservation of time for maximum profit making and skin contamination.

Keywords: Petrol, toxic, methaemoglobin, benzene, blood dyscaria

INTRODUCTION

Nigeria is the 6TH largest producer of oil in the world and it is endowed with more gas reserves than oil (Aston-Jones, 1998; NNPC, 2011). In 2011, total crude oil and condensate production was 866,245,232 barrels, giving a daily average of 2.37 mmb/pd, while in the gas sector, a total of 2,400.40 Billion Standard Cubic Feet(BSCF) of Natural Gas was produced by sixteen companies; of the quantity produced, 1,781.37 BSCF (74%) was utilized, while 619.03BSCF (26%) flared (NNPC, 2011).

Despite the effort of the government in restructuring the petroleum industry, most of the refineries in the country are working at a sub-optimal level due to constant vandalization of oil pipelines, oil spillage, poor maintenance, illegal refineries and other illegal activities. During these processes the handlers of crude oil and its products are exposed to various cytotoxic chemicals that may induce anaemia and other pathologic effects. Refined products

from these activities are traded by touts or peddlers including women and children without observing any safety measures in most local communities in Nigeria. The unavailability of the products in the organized market and periodic fuel price hikes further promotes patronage of these 'black markets'.

Occupational exposure to petroleum has 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).

Gasoline or petrol is a clear but slightly yellowish liquid mixture which is primarily used as fuel in internal combustion engine (Ron, 2004). Petrol comes from crude oil and it is a mixture of organic hydrocarbon. It consists mostly of aliphatic hydrocarbons (HCs) obtained by fractional distillation of petroleum, enhanced with isooctane or the aromatic HCs like toluene or benzene to increase its octane rating. The bulk of a typical gasoline consists of HCs with below 4-12 Carbon atoms (Collins, 2007), molecule commonly referred to as C₄-C₁₂. Many of these HCs are hazardous substances and are regulated in the United States by the Occupational Safety and Health Administration. PMS contains volatile organic compounds like benzene and the content is regulated to 6% to 8% of the content of PMS in Nigeria (Rowat, 1998), and between 1%(v/v) to 5% in the USA and Europe (Menkes *et al.* 1997; Wallace, 1996).

Although, leaded petrol is no longer available, unleaded however still contains small traces of lead. The material safety for unleaded gasoline shows at least 15 hazardous chemicals occurring in various amounts, including benzene (up to 5% by volume, toluene (up to 35% by vol), trimethylbenzene (up to 7% by vol), naphthalene (up to 1% by vol), trimethylbutyl ether (MTBE) up to 18% by vol, in some states and about 10 others. Benzene and lead content of PMS poses health risks for everyone (Dean, 1985). Exposure to benzene causes various cancers, skin, lungs and kidney damage and death upon exposure to very high concentrations (Rinsky *et al.*, 1981). Others include autoimmunity (regressing to diseases of the joint e.g. Rheumatoid arthritis), and Central nervous system involvement. Others include, mucous membrane irritation. However, it is worthy to note that there is acute and chronic toxicity (Carballo *et al.*, 1995).

After acute inhalation of the PMS fumes, they are rapidly absorbed into the blood and distributed throughout the body resulting in mucous membrane irritation, neurological and other symptoms due to respiratory failure (Hunter, 1966; Carballo *et al.*, 1995). Chronic toxicity on the other hand has been reported to cause Bone-Marrow (BM) depression, aplastic and leukaemia, cardiac abnormalities, heart attack and other cancer of the lungs, brain and stomach (Travis *et al.*, 1990).

High doses however can result into developmental retardation of foetus. It poses a higher risk in children, unborn babies and women of child bearing age. Children exposed to high levels of lead after birth may develop behavioural and learning problems, slow growth and finally hearing loss (EPA, 2009). Exposure to high levels of lead during pregnancy contributes to miscarriages, preterm birth, low birth weights and developmental delays in infants (Kunitz and Levy, 1974).

Lead, when absorbed by the bone and fat in the brain can remain for 10 years. Over this time, it is released back into the body after sniffing stops (Bass, 1970). Thus accounting for the social and behavioural problems often associated with workers handling PMS (Nurcome *et al.*, 1970). Further study revealed that most women who are exposed to higher than average levels of air pollution have healthy babies. However, studies from the United States and other countries suggest that pregnant women exposed to high levels of certain air pollutants may be slightly more likely than pregnant women living in less polluted areas to have a premature or small-for-age children (Stillerman *et al.*, 2008).

Continuous exposure of PMS vapour has been reported in both experimental animal and human to cause harmful effects on the reticuloendothelial system by destroying or inhibiting the haematopoietic components of the BM and induction of leukaemia during occupational exposure (Wixson and Brown, 1992). In a recent publication, benzene when inhaled into the body produces methaemoglobin (methb) which differs from Haemoglobin based on the absence of electron that is needed in the formation of a strong bond with oxygen needed for oxygen transport. Increase in level of methb results in symptoms like shortness of breath, palpitation, anxiety, and confusion. PMS, also causes a reduction in Haematological indices which become chronic with

prolong exposure. The measurement of serum methb is an indirect method of its evaluation of this form of poisoning (Okoro *et al.*, 2006).

Objective of the study

The purpose of this research is to investigate the potential risk associated with exposure of non-pregnant women of child bearing age selling PMS (WSP) in Okada-Ore Road, Edo State, Nigeria using batteries of haematological parameters and level of MetHb as an indirect blood biomarker of exposure to benzene in PMS. This study is worthwhile because no studies have been reported on blood dyscrasia among this study group.

Subject and Methods

Women recruited to this study are age matched for control and study population. The study was conducted between January, 2008 to January, 2009. The length of the work in their business was taking as the duration of exposure to PMS. This cross-sectional survey was carried out among women of child bearing age selling PMS in Okada- Ore Road as test subject and apparently healthy, non PMS seller as control population. 100 women each for both test and control population within the age 18-34 years were recruited into the study after an informed consent through an oral interview and structured questionnaire. 5Milliliters of the venous blood from the ante-cubital vein was collected into an EDTA Bottle using standard methods. It was stored in a cool pack before taken to the laboratory for analysis. Blood sample were analysed within 24hours of collection. Haematological parameters were determined according to method of Dacie and Lewis, 1984. Analysis of Met-Hb were done using a method according to Evelyn and Malloy, 1938.

RESULTS

Table 1: Showing the Age distribution of Subject (Women Selling Pms) and Control (women not selling PMs)

AgeGroup(yrs)	Age (years)	
	PMS Seller (%)	Control / Non-Seller (%)
18-21	15(15.0)	15(15.0)
22-25	40(40.0)	40(40.0)
26-29	35(35.0)	35(35.0)
≥30	10(10.0)	10(10.0)
Total	100(100.0)	100(100.0)

Table II: Blood cell count in relation to year of exposure to PMs

Years of Exposure	WBC ($10^9/L$)	Platelets ($10^9/L$)	RBC ($10^{12}/L$)
≤ 1	4.20±0.10	260±10.0	3.80±0.05
2-3	3.60±0.20	230±20.0	3.65±0.05
3-4	3.40±0.15	160±10.0	3.20±0.05
≥ 5	2.80±0.10	130±10.0	3.00±0.05
Control	4.60±1.50	285±30.0	4.70±1.50

Table III: Showing Hbg/dl, PCV and Red cell indices with year of exposure to PMs

Exposure (Yrs)	Hbg/ dl	PCV (L/L)	Red cell count	MCV (fl)	MCHC (g/dl)	MCH (pg)
≤ 1	13.20±0.06	0.39±0.01	3.80±0.05	102.63	33.85	34.74
2-3	11.30±0.12	0.36±0.02	3.65±0.05	98.63	31.39	30.96
3-4	10.60±0.14	0.34±0.01	3.20±0.05	106.25	31.18	33.13
≥ 5	9.46±0.12	0.30±0.01	3.00±0.05	100.00	31.53	31.53
Control	13.50±0.01	0.42±0.01	4.70±1.50	89.36	33.33	28.72

Normal value Hbg/dl 12g/dl (women non-pregnancy); MCV(Men and women 92±9fl), MCH 29.5±2.5Pg;MCHC (33±15g/dl) as expressed as a mean±2SD(95% range).(Evatt et al,1983)³⁴

Table IV: Exposure to PMS and percentage (%) of Met Hb

Exposure (years)	Test subject Met Hb %
≤1	2.4
2-3	2.9
3-5	8.0
≥5	10.5

Normal Range - Met Hb < 2.0%

DISCUSSION

In the study of the acute toxicological effects of diesels and crude oil that contains benzene in an experimental animals, it was observed that an increase in the dose of fuel administered into the animals caused a dose dependent decrease in Hb and PCV (Dede and Kagbo, 2002). Haematological changes in women of child bearing age expose to PMS have not been well assessed in Nigeria. Observation from our study revealed that Hb and all other haematological parameters decreases with increase level of exposure resulting in progressive anemia. This observation is married to constant and repeated unproductive exposure to PMS thereby eliciting a gradual iron depletion in the iron store via inhalation of fumes resulting is serious public health concern (Hotz and Lauwerys, 1992).

A study by Akintonwa and his colleagues revealed that chronic exposure to petroleum products may have deleterious effects on the health of the filling station attendants studied. They however, concluded that exposure may have little or no effect on the red and white cell but cause a significant decrease of 43% in platelet count as compare with the control group (Akintonwa et al., 2005). Their finding is contrary to a study conducted in the same group of subject in southern Nigeria with significantly higher effect on red, white, and platelet cell as compared with control (Hayes et al., 1997). These finding equally agree with result obtained from similar work conducted by (Dede and Kagbo, 2002; Udonwa et al., 2009). From our study also, it was equally observed that women exposed to petroleum had a significant higher Met Hb than control group.

Other Study confirms that most women who are exposed to higher than average levels of air pollution have healthy babies, However, studies, from the united states and other countries suggest that pregnant women exposed to high levels of certain air pollutant as benzene may be slightly more likely than pregnant women living in less polluted areas to have a premature or small -for -gestational-age baby (Stillerman et al., 2008; Wilson and Schwarzman, 2009).

From other similar work done in southern Nigeria in a separate group handling PMS, it was reported that the major route of exposure to PMS is by mouth suction due to conservation of time for maximum profit making and skin contamination 29,33. In develop countries as USA exposure has been limited to the use of rubber hood over the delivery pump to use of "self service" at the stations.. Mohorovic, 2003; Wilson and Schwarzman, (2009) in their findings observed in its chronic toxicity summary that the inhalation reference exposure level of 60ug/m³ causes a critical effect on lowering red and white cell counts in occupationally exposed humans. The hazard index targets include haemopoietic system, development, and nervous system (Aksoy et al., 1972).

Observation from our study stained thin film of subject exposure for and above 5years showed a leucoerythroblastoid picture with decrease total leucocyte count this finding is similar in the work done by Carballo et al., 1995. The red cell indices decreases with increase in years of exposure.Red blood picture of subject within 1-3years of exposure shows that of dimorphic blood picture with microcytic normocytic hypochromic cells as compared with the pattern of result of red cell indices .Platelet count decrease with level of Subjects above 5years of exposure resulting in mild thrombocytopenia with few blast.

This finding is similar to recent report by Wixtron and Bown, 1992; Aksoy et al., 1972 has demonstrated the Impairment of immune function and/or various anaemia may result from the haematotoxicity with hematologic lesion and bone marrow involvement leading to peripheral lymphocytopenia and / or pancytopenia following chronic exposure (Aoyama, 1986).These lesions can lead to the induction and or development of leukaemia years after apparent exposure (Aoyama, 1986).

This study suggested increased exposure to PMS fumes among its seller while the primary route is oral suction and skin contact. Met Hb is a useful biomarker in determining the level of exposure. In Nigeria, PMS dispensing station is scarcely inadequate in most rural and some urban-rural communities. We therefore, recommend enlightenment campaign to educate PMS seller on negative health implication of exposure to fumes. Close regulatory monitoring of individual selling petroleum and petroleum products is also very important. This can be achieved by mandatory provision and enforcement of the use of protective fittings and or adequate stationing of filling station with dispenser in local communities to reduce the fumes inhalation.

Determination of Methhaemoglobin

About 0.2milliter of blood was lysed in a mixture of 4ml of phosphate buffer (pH6.8) and 6milliter of non-ionic detergent .The lysate was then divided into two equal volumes in separate tubes (A and B) .The absorbance of 1 part was read spectrophotometrically at 630nm (Tube A) and recorded as D1 .The absorbance spectrum of met-Hb exhibits a small characteristic peak of 630nm.The addition of cyanide eliminates the peak and the decrease in absorbance is proportional to the metHb concentration. Addition of ferricyanide measures total Hb after conversion to metHb. One drop of potassium ferricyanide was added to the 1part A, mixed and absorbance recorded as D2.One drop of potassium cyanide was added to B, mixed and allowed to stand for 5 minutes and absorbance recorded as D3.One drop of potassium cyanide was added to B, mixed and read as absorbance D4.Four millilitre of phosphate buffer plus 6millilitre non-ionic determine was used as blank.

Result was expressed as MetHb (%) = $\frac{D1-D2}{D3-D4} \times 100$

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