Vol. 19(6), pp. 70-77, September 2020
DOI: 10.5897/AJMHS2020.0106
Article Number: 2FF265A64719
ISSN: 2384-5589

http://www.academicjournals.org/AJMHS

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African Journal of Medical and Health Sciences

Full Length Research Paper

Levels of some reproductive hormones, cadmium and lead among fuel pump attendants in Benin City, Nigeria

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Received 10 May 2020; Accepted 20 July 2020

Infertility is a common public health challenge in Nigeria. The causes of infertility in both males and females are multifactorial, and the contributions of endocrine abnormalities had been reported from both the Northern and Southern regions of Nigeria. The effect of occupational exposure on endocrine levels among petrol pump attendants has not been sufficiently documented in our setting. This study evaluated the levels of reproductive hormones, blood cadmium, and lead among fuel pump attendants in Benin City. A total of 60 petrol attendants and 30 healthy non-occupationally exposed subjects were recruited for the study. A blood specimen was collected and evaluated for follicle-stimulating hormone (FSH), luteinizing hormone (LH), progesterone, estrogen, and testosterone as well as blood cadmium and lead using ELISA and Atomic Absorption Spectrophotometer (AAS) respectively. Serum levels of FSH, LH, estradiol, progesterone and testosterone were significantly lower among fuel attendants than non-occupationally exposed control subjects. The mean levels of blood cadmium and lead were significantly higher among fuel attendants than controls. Reproductive hormone levels significantly correlated with the duration of exposure to petrol fumes, FSH (r=-0.50, p=0.001), LH (r=-0.52, p=0.001), estradiol (r=0.32, p=0.009), progesterone (r=-0.35, p=0.005) and testosterone (r=-0.48, p=0.001). Blood cadmium and lead correlated negatively with reproductive hormones except between lead with estradiol (r=0.14, p=0.276) which was positively correlated. The levels of measured reproductive hormones were significantly lower, while cadmium and lead were significantly higher in fuel attendants than non-occupationally exposed control subjects. Exposure to petroleum fumes may be a risk factor and may be associated with reproductive hormone abnormalities. Personal protective devices should be worn by petrol attendants in order to avoid the adverse consequences of the observed abnormalities.

Key words: Sex hormones, fuel pump attendants, occupational exposure.

INTRODUCTION

Infertility is a public health challenge, and the prevalence among Nigerians is increasing (Owolabi et al., 2013).

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Occupational exposure to gasoline, which contains potential toxicants and endocrine disruptors, could act as predisposing factors to infertility in exposed individuals. It is not completely clear whether acute or chronic exposure has the same effect on sex hormones. Fuel pump attendants in most petrol stations do not wear protective devices when on duty. The gasoline contains varieties of contaminants, which include benzene, toluene, ethylbenzene, and xylene (BTEX), cadmium, and lead that could cause an overwhelming effect on humans as a result of exposure to these chemicals without protective apparatus (Garshick et al., 2008).

Crude oil is made of different hydrocarbons, and the chemical composition varies according to the geological formulation (Edwards et al., 1989). The refined products of crude oil include petrol, kerosene, diesel, and lubricants, but petrol and kerosene are the mostly used derivatives (Kato et al., 1996). These fractions contain aromatic, aliphatic, branched saturated and unsaturated chains of hydrocarbons at different concentrations (Uboh et al., 2009). The BTEX, cadmium, and lead are the major toxic constituents of petroleum products due to their high coefficient diffusion rate into the bloodstream (Azari et al., 2012). Fuel pump attendants are exposed through inhalation, ingestion, and dermal routes because of the volatile and lipophilic nature of petroleum compounds (Cecil et al., 1997). Increase exposure to various constituents of hydrocarbon occurs in the society due to domestic and industrial use of petroleum products. These exposures can be accidental or intentional (Edminster, 1985; Cairney et al., 2002). Previous studies have shown that emission radiations can be detrimental to health and also reduce the quality of air (Bruce et al., 2002; Rekhadevi et al., 2010; World Health Organization, 2012; Singh et al., 2013).

According to the United States Environmental Protection Agency (USEPA), “BTEX are explosive aromatic compounds found in petroleum products, such as gasoline and diesel fuel” (USEPA, 2010). Moreover, evidence has shown that BTEX in gasoline is reproductive toxicants. Also, the relationship between the level of exposure and its adverse effect on reproductive functions such as altered fertility, congenital malformation, and chromosomal abnormalities have been tested and documented in experimental animals (Ugwoke et al., 2005). The ovaries and testes are known target organs damaged by these toxicants (BTEX, cadmium and lead), although their actions on other endocrine organs such as the hypothalamus, pituitary, adrenals, thyroid, parathyroid, and pancreas are documented (Mattison et al., 1990; Verma and Rana, 2009). Furthermore, benzene and its derivatives affect the luteal functions of female workers in petrochemical industries (Chen et al., 2000). Epidemiological studies that assessed the health risks of fuel pump attendants indicate that the effects of these volatile organic compounds can result in cancers, congenital defects, reproductive disorders, spontaneous and respiratory diseases (Wiwanitkit, 2008; Rushton et al., 2014). The objective of this study was to assess the level of awareness and use of a personal protective device, evaluate the serum levels of reproductive hormones, blood cadmium and lead in fuel pump attendants and correlate with duration of exposure in Benin-City.

MATERIALS AND METHODS

Study design and population

This is a cross-sectional study of occupationally exposed fuel pump attendants. The fuel pumps attendants who were occupationally exposed for one year and above; those that complied with the inclusion criteria, were recruited using a simple random stratified sampling technique. The study group consists of 60 participants (45 males and 15 females), while 30 control subjects made up of 21 males and 09 females were enrolled in the study. They comprise individuals from different ethnic groups, complexion, body mass index, various levels of educations, married and single were recruited in the study.

The study was designed to evaluate the levels of reproductive hormone: FSH, estradiol (E2), luteinizing hormone LH, progesterone and testosterone, and blood cadmium and lead in fuel pump attendants. We selected commercial petrol stations as study sites based on their geographical location to represent the entire study area. The study was carried out between May to October 2017.

The sample size was determined using the sample size determination formula for Health studies (Lwange and Lemeshow, 1991) and prevalence of exposure to petrol fumes of 96.7% (Johnson and Bassey, 2014). Therefore a minimum of 60 participants and 30 non-occupationally exposure healthy subjects were enrolled for the study.

Ethical consideration

Ethical clearance was obtained from Edo State Ministry of Health, Benin City (ethical code HM1208/157, dated 8th June 2017). Permission was obtained from the Depot Managers of various petrol stations and informed consent obtained from individual subjects before the commencement of the study.

Inclusion and exclusion criteria

The inclusion criteria were fuel pump attendants aged between 18 and 45 years, non-pregnant or breastfeeding, non-user of oral contraceptives, non-smokers, and with no history of infertility, menstrual disorders, pelvic inflammatory disease before employment as fuel pump attendants and moderate physical activity. Other chronic metabolic diseases that are confounding with the study subjects were excluded from this study.

Data collection

Qualitative data were collected using a semi-structured, self-administered questionnaire. The questionnaires consisted of three (3) sections with open-ended questions and probes aimed at exploring the socio-demographic profile: Section A (Socio-demographic characteristics), Section B (awareness and use of personal protective device), and Section C (Medical/family history). The questionnaire was distributed among the fuel pump attendants in petrol stations.

Sample collection and preparation

Blood samples were collected aseptically from the ante cubital vein of the fuel pump attendants, and, healthy non-occupationally exposed control subjects in the morning hours of the day with sterile disposal needle and syringe, and dispensed into an anticoagulant container (prescreened for cadmium and lead by vial assay) and plain containers. The sample in the plain container was allowed to clot at 2-8°C, and after clot retraction, it was centrifuged at 3000 rpm for 10 min. The plasma was separated and stored properly in labeled containers at -20°C for hormonal assay, and the whole blood was stored at 2 to 8°C for a maximum of one week for cadmium and lead analyses.

Measurement of reproductive hormones

The reproductive hormones (FSH, LH, estradiol, testosterone, progesterone) were assayed by the ELISA technique (Diagnostic Products Monobind Inc. Lake Forest, CA 92630, U.S.A.).

Principle of Elisa technique

The antigen present in the patient sample and calibrator bind to the coated antibody. The antigen-antibody complex reacted with enzyme conjugate, which converted the substrate into a colored solution. The intensity of the color change is proportional to the concentration of enzyme-antibodies present in the samples, which was read at 450 nm.

Measurement of cadmium and lead

The blood cadmium and lead were assayed using Atomic Absorption Spectroscopy (Buck Scientific Model VGP-210, Germany).

Principle of atomic absorption spectroscopy (AAS)

Atomic absorption spectroscopy is based on the principle that when a beam of electromagnetic radiation passed through a substance, the radiation may either be absorbed, which leads to an increase in the energy of the molecule, where the electron undergoes excitation to higher ground level. The energy gained by the molecule is directly proportional to the wavelength of radiation.

Procedure

Exactly 2 mL of each sample was digested with a mixture of 4 mL HNO_3 and H_2SO_4 (ratio 4:1) and heated for 4 h slightly in a water bath at a temperature of 80°C to ensure complete digestion. The digested samples were then taken for A.A.AS analysis. The metal contents of the digested samples were determined by aspiration (air/acetylene flame) using atomic absorption spectroscopy (Buck Scientific Model VGP-210, Germany) at appropriate wavelengths of 217.0 and 228.8 nm for Pb and Cd, respectively. The limits of detection (LOD) for the blood metal levels were as follows: Cd, 0.2 µg/L and Pb, 0.3 µg/dL, respectively (AOAC, 1990). Cadmium and lead standards were adequately prepared in order to check the reliability of the data. A standard sample of the element was diluted to obtain serial dilutions of each sample and was used to calibrate and standardize the electrothermal atomic absorption spectrophotometer before running the analysis, and a graph was generated. Before use, all volumetric polyethylene (including the autosampler cups) and glass material were cleaned by soaking in 20% (v/v) HNO_3 for 24 h. The materials were finally rinsed with several washes of Milli-Q® water and dried in a polypropylene container. Certified reference materials from (Le Centre de toxicologie du, Quebec) were analyzed.

Statistical analysis

Results obtained are presented as mean ± standard deviation (SD). Data from the study were analyzed using SPSS version 23.0 software. The comparison of mean values of measured parameters between fuel pump attendants and controls was performed using unpaired Student's t-test, Chi-square and Pearson's correlation coefficient was used to test the relationship between duration of exposure and effect on sex hormones. The statistical significance level was set at $p<0.05$.

RESULTS

Table 1 shows the socio-demographic variables of the study participants. The age range, gender, duration of exposure, educational status, body mass index (BMI), ethnicity ($p<0.001$), use of personal protective device ($p<0.013$) and awareness of adverse effect ($p<0.002$) were significantly different between fuel pump attendants and control subjects. Table 2 shows the comparison of blood cadmium, lead, and reproductive hormones among male fuel attendants and male control subjects. It was observed that the reproductive hormone levels were significantly lower ($p<0.001$) among male fuel attendants than male control subjects. Similarly, the blood cadmium, lead, and sex hormones levels (except serum testosterone) were significantly lower among female fuel attendants than female control subjects (Table 3).

Table 4 shows the correlation between reproductive hormones and duration of exposure. There was a significant negative correlation between the duration of exposure and reproductive hormones of male fuel attendants ($p<0.05$). In the same vein, blood cadmium and lead correlated negatively with measured sex hormones among fuel pump attendants, except estradiol with lead, which was not statistically significant (Table 5).

DISCUSSION

Nigeria belongs to the infertility belt of sub-Saharan Africa, and infertility has assumed a public health

Table 1. Demographic distribution of study participants and control subjects.

Demographic variable	Total (n=90)	Fuel attendants (n=60)	Control subjects (n=30)	X^2	p-value
Age (years)					
18-25	56(62.2%)	31(51.6%)	25(83.3%)		
26-35	20(22.2%)	15(25.0%)	5(16.6%)		
36-40	8(8.8%)	8(13.3%)	0(0.0%)	71.60	P=0.001
40-above	6(6.6%)	6(10.0%)	0(0.0%)		
		Sex			
Male	66(73.3%)	45(75.0%)	21(70.0%)	19.60	P=0.001
Female	24(26.6%)	15(25.0%)	9(30.0%)		
Marital status					
Single	52(57.7%)	32(53.3%)	20(66.6%)	2.18	P=0.140
Married	38(42.2%)	28(46.6%)	10(33.3%)		
Duration of exposure (years)					
<1		10(16.6%)	0(0.0%)		
1-5		30(50.0%)	0(0.0%)		
6-10		8(13.3%)	0(0.0%)		
11-15		6(10.0%)	0(0.0%)		
16-20		4(6.6%)	0(0.0%)	67.11	P=0.001
20-above		2(3.3%)	0(0.0%)		
Educational status					
Primary	5(5.5%)	5(8.3%)	0(0.0%)	62.0	P<0.001
Secondary	64(71.1%)	44(73.3%)	20(66.6)		
Tertiary	21(23.3%)	11(18.3%)	10(33.3%)		
BMI (kg/m^2)					
Underweight	3(3.3%)	3(5.0%)	0(0.0%)		
Normal	42(46.6%)	22(36.6%)	20(66.6%)	45.53	P<0.001
Overweight	32(35.5%)	23(38.6%)	9(30.0%)		
Obese	13(14.4%)	12(20.0%)	1(3.3%)		
Ethnicity					
Benin	81(90.0%)	51(85.0%)	30(100.0%)	275.78	P<0.001
Igbo	3(3.3%)	3(5.0%)	0(0.0%)		
Yoruba	2(2.2%)	2(3.3%)	0(0.0%)		
Hausa	1(1.1%)	1(1.6%)	0(0.0%)		
Ijaw/Itsekiri	3(3.3%)	3(5.0%)	0(0.0%)		
Use of PPD					
Yes	17(18.8%)	17(28.3%)	0(0.0%)		
No	43(47.7%)	43(51.6%)	0(0.0%)		P<0.013
No response	30(33.3%)	0(0.0%)	30(100%)	21.51	
Awareness of adverse effect					
Yes	22(24.4%)	16(26.6%)	6(20.0%)		P<0.002
No	68(75.5%)	44(73.3%)	24(80.0%)		

PPD= Personal protective device; BMI = Body mass index.

Table 2. Comparison of blood cadmium, lead and sex hormone levels between male fuel attendants and male control subjects (Mean±SD).

Variable	Male fuel attendants (N=45)	Male control subjects (N= 21)	P-value
Cd (ug/dl)	7.94±0.47	1.28±0.31	P=0.001
Pb (ug/dl)	1.78±0.13	0.77±0.05	P=0.001
FSH (mIU/ml)	1.68±0.29	4.98±0.47	P=0.001
LH (mIU/ml)	2.76±0.43	9.26±1.65	P=0.001
E2 (pg/ml)	3.12±0.47	5.59±0.35	P=0.001
Prog (ng/ml)	0.25±0.06	0.55±0.11	P=0.013
Testo (ng/ml)	2.61±0.29	6.77±0.52	P=0.001

Cd- Cadmium; Pb- lead; FSH- follicle-stimulating hormone; LH-Luteinizing hormone; E2-Estradiol; Prog –Progesterone; Testo-Testosterone.

Table 3. Comparison of blood cadmium, lead and sex hormone levels between female fuel attendants and female control subjects (Mean±SD).

Parameter	Female fuel attendants (n=15)	Female control subjects(n=09)	P-value
Cd (ug/dL)	3.48±0.38	0.10±0.01	0.001
Pb (ug/dL)	1.47±0.25	0.59±0.04	0.014
FSH (mIU/mL)	5.42±0.01	7.24±0.003	0.001
LH (mIU/mL)	3.12±0.12	4.56±0.20	0.001
E2 (pg/mL)	51.3±0.18	60.93±0.20	0.001
Prog (ng/mL)	9.21±0.21	11.62±0.40	0.001
Testo (ng/mL)	0.65±0.25	0.93±0.07	0.418

Cd- Cadmium; Pb, lead; FSH, follicle stimulating hormone; LH, Luteinizing hormone; E2, Estradiol; Prog, Progesterone; Testo, Testosterone.

Table 4. Correlation of duration of exposure with hormone profile among the study participants.

Correlation	R-value	P-value
Duration/FSH	-.50	0.001
Duration/LH	-0.52	0.001
Duration/estradiol	-0.32	0.005
Duration/testosterone	-0.48	0.001
Duration/cadmium	0.53	0.001
Duration/lead	0.49	0.001

challenge. The causes of infertility in both males and females are multifactorial and the contributions of endocrine abnormalities have been reported (Emokpae et al., 2007). Infertility confers social implications on affected families and couples (Owolabi et al., 2013). This research determined the effect of occupational exposure of gasoline on sex hormones and blood levels of cadmium and lead in fuel pump attendants. The significantly lower levels of FSH and LH among fuel pump attendants than control subjects could be attributed to inhalation of volatile organic compounds such as BTEX and fuel additives (lead and cadmium) which disrupt the function of hypothalamic-pituitary glands. This may affect the trophic hormone secretion as a result of the downregulation of gonadotropin-releasing hormones (GnRH), GnRH receptor and pituitary-1 receptor mRNA level (Miyamoto et al., 2012, Priskorn et al., 2012, Akintunde et al., 2012). This observation is in agreement

Table 5. Correlation of blood cadmium and lead with measured sex hormones.

Correlation	R-value	P-value
Cadmium/FSH	-0.37	0.002
Cadmium/LH	-0.39	0.001
Cadmium/estradiol	-0.28	0.021
Cadmium/progesterone	-0.37	0.02
Cadmium/testosterone	-0.35	0.001
Lead/FSH	-0.29	0.015
Lead/LH	-0.36	0.003
Lead/estradiol	-0.14	0.279
Lead/progesterone	-0.29	0.019
Lead/testosterone	-0.24	0.022

with a previous study (Kim et al., 1998).

The observed significantly higher mean level of cadmium among fuel pump attendants is in agreement with a previous study (Bolawa et al., 2014). The mean plasma cadmium level observed in this study was however lower than the 48.8±5.1 µg/dl reported by Ibeto and Okoye (2009) for blood cadmium level of occupationally exposed individuals within Enugu, Nigeria. It was, however, higher than the 2.29±0.12 µg/dl reported by Musa et al. (2011) for blood cadmium level in occupationally exposed individuals in Zaria, Nigeria. The mean plasma cadmium level for both the occupationally exposed (fuel attendants) and non-occupationally exposed subjects in this study were higher than the WHO's permissible range of 0.03 to 0.12 µg/dl of cadmium (WHO, 1996). The high blood cadmium level observed in this study may be due to accumulation in the body, which could lead to disruption of steroidogenesis by interfering with the biosynthesis of androgens, oestrogen and progesterone (Georgescu et al., 2011). Cadmium may bind both oestrogen and androgen receptor through an interaction with the hormone-binding domain receptor.

In the same vein, the significantly higher mean level of lead among occupationally exposed participants than non-exposed subjects is in agreement with previous studies elsewhere (Ogunsola et al., 1994; Babalola et al., 2005). The mean value reported in this study is lower than 48.5±9.08 µg/dl recorded for occupationally exposed workers in Abeokuta, Nigeria (Babalola et al., 2005) and 18.1±6.4 and 10.2±2.7 ug/dl reported for occupationally exposed traffic wardens in Lagos and Ife, Nigeria, respectively (Ogunsola et al., 1994). It is also lower than 12.3 µg/dl reported for the male population in Ibadan, Nigeria (Omokhodion, 1994). Because the half-life of lead in the blood is 35 days, the mean blood level observed in this study may be suggestive of recent lead exposure. The continuous use of leaded gasoline for vehicles in Nigeria may have contributed to the relatively high blood level of lead among fuel attendants when compared with controls. Poor usage of personal protective devices observed among fuel attendants in this study (28.3%) may also contribute significantly to the level of lead observed in their blood. It is important to note that the blood lead levels observed in the non-occupationally exposed subjects in this study and several other studies are indicative of the extent of environmental lead pollution in Nigeria (Ogunfowokan et al., 2002; Orisakwe, 2009; Galadima and Garba, 2012). This is of serious concern as it implies that many Nigerians are exposed to lead and other toxic metals from the environment irrespective of their occupation. Toxic metals including lead act by altering the reproductive hormonal axis (by inhibiting adrenal steroidogenesis, progesterone, testosterone, and 17β-estradiol) and the hormonal control on spermatogenesis, rather than by a direct toxic effect on the seminiferous tubules of the testes as well as potent enzyme inhibitors responsible for the synthesis of male and female hormones (Mohsen et al., 2011 Sharma et al; 2012; Chikezie et al., 2017). Sirotkin et al. *(*2012) reported that petrochemical industrial environmental contaminants such as lead and cadmium could interfere with the release of the ovarian hormone.

In this study, statistically significant negative correlation was observed between cadmium, lead and the reproductive hormones. This indicates that exposure to cadmium and lead could adversely affect the sex hormones levels probably by causing hypothalamic-pituitary axis disruption or by directly affecting spermatogenesis and ovulation, resulting in impaired semen quality and infertility (Wyrobek et al., 1997, Jurasoviæ et al., 2004). Other authors have reported a decline in semen quality associated with both lead and cadmium (Eibensteiner et al., 2005; Telisman et al., 2007). Another study reported that association exists between impaired sperm motility, cadmium, and lead concentrations in seminal fluid, which indicate that presence of lead and cadmium in the reproductive tract of men attending infertility clinics may be related to a moderate alteration of their critical parameters (Mendiola, 2011; Emokpae and Adobor, 2015).

In this study, it was observed that there was a significant negative correlation between reproductive hormone levels and duration of exposure. This could be attributed to the adverse effects of a toxic solvent constituent of gasoline (BTEX, cadmium, and lead) and the dose of exposure on the endocrine system over a longer duration and repeated absorption through inhalation and other routes. This is in agreement with the previous study (Sexton et al., 2007). These authors reported that exposure to benzene above the threshold limit value (T.L.V.) due to prolonged exposure and absorption was associated with the higher adverse reproductive effect, which was seen in this study. Results in this study illustrate that long term exposure to gasoline fume can lead to increase risk which is a function of high blood/air coefficient as well as rapid perfusion and absorption rate of BTEX, cadmium, and lead, exposure concentration, genetic and ethnicity (Sexton et al., 2007). The significant effect of gasoline fume on reproductive hormones throughout the exposure time could be associated with the generation of reactive oxygen species (R.O.S.), which could lead to altered structure, functions, and multi-system toxicity (Ekpenyong and Asuquo, 2017).

Conclusion

Sex hormone levels were significantly lower, while blood cadmium and lead were significantly higher among fuel pump attendants when compared with non-occupationally exposed controls. The low levels of reproductive hormones and higher levels of blood cadmium and lead correlated with duration of exposure. This showed that occupational exposure to these endocrine disruptors may have led to the alteration in the pituitary and gonadal hormone levels. Prolong exposure to petroleum pollutants may be a predisposing factor to the impairment of the endocrine system. It can cause a wide variety of toxicological effects on body tissues as well as biochemical dysfunction that constitute a serious health hazard to humanity. It is therefore suggested that the petrol pump attendants should wear personal protective devices when on duty to avoid direct inhalation and transdermal absorption of the petrol fumes. The clinical application of this finding may be of importance to the health management of workers in the petroleum industry.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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