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OCCUPATIONAL EXPOSURE TO HEAVY METALS: ASSESSING IMMUNE AND LIVER TOXICITY IN PETROLEUM INDUSTRY WORKERS

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Abstract: This study investigates the impact of exposure to heavy metals on the health of workers in the petroleum industry. In this study forty participants in four groups were involved. Group 1 was the control, while groups two, three and four were samples collected from mechanic workers, filling station attendance and artisan petroleum handlers respectively. Blood samples were collected and analyzed for heavy metals, immunological parameters and liver function parameters. The results revealed that the concentration of the various heavy metals was highest in artisan petroleum handlers, followed by mechanic workers and filling station attendants. The heavy metal concentration was significantly lowest in the control group. Also from the result, the immunological parameter was highest in artisan petroleum workers followed by the mechanic workers and filling station attendance. The control group has the lowest level of immunoglobulin level. The result further revealed that the level of liver enzymes was significantly low in control group followed by the filling station attendants, the mechanic workers and finally the artisan petroleum handlers respectively.

Keywords: Immunological, Petroleum, Blood, Heavy metal, Liver function parameters

Introduction

Heavy metals are known for their persistence in the environment, meaning they do not degrade or break down easily. They can accumulate in living organisms through bioaccumulation and biomagnification, leading to higher concentrations in top predators, including humans. The primary sources of heavy metal pollution include natural sources like volcanic eruptions and weathering of metal-bearing rocks, as well as anthropogenic activities such as mining, smelting, industrial production, and the use of metal-containing products like batteries and pesticides (Jaishankar et al., 2014). In the context of occupational exposure, industries such as mining, metalworking, and petroleum refining are significant contributors to heavy metal exposure. Workers in these industries are at higher risk due to direct contact with metals during extraction, processing, and waste handling. For example, petroleum refining involves processes that can release heavy metals like vanadium, nickel, and lead into the air and water, thereby increasing the risk of exposure for industrial workers (Ite et al., 2018). The petroleum industry plays a crucial role in global economic development, driving significant advancements in energy production. However, the industry also presents substantial occupational hazards, particularly for workers exposed to toxic substances, including heavy metals such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) (Jaishankar et al., 2014). These metals are commonly released during various stages of petroleum extraction, refining, and processing, posing a potential health risk to industrial workers who are in constant contact with them. Prolonged exposure to these metals in industrial environments can lead to their accumulation in human tissues, disrupting vital biological functions. In the petroleum industry, workers may be exposed to these metals through inhalation of contaminated air, ingestion of polluted water or food, and dermal contact with equipment or surfaces contaminated by industrial by-products. In the context of occupational exposure, industries such as mining, metalworking, and petroleum refining are significant contributors to heavy metal exposure. Workers in these industries are at higher risk due to direct contact with metals during extraction, processing, and waste handling. For example, petroleum refining involves processes that can release heavy metals like vanadium, nickel, and lead into the air and water, thereby increasing the risk of exposure for industrial workers (Ite et al., 2018). The liver is a primary organ responsible for detoxifying harmful substances, including heavy metals. However, chronic exposure to high levels of these metals can lead to hepatotoxicity, resulting in liver damage and dysfunction. Studies have shown that heavy metals such as cadmium and mercury can induce oxidative stress, impairing liver cells and contributing to conditions such as fatty liver disease, fibrosis, cirrhosis, and even hepatocellular carcinoma (Satarug et al., 2010). Heavy metal toxicity not only affects the liver but also impairs the immune system. These metals are known to disrupt immune regulation by altering the function of immune cells such as macrophages, lymphocytes, and neutrophils. This disruption can lead to immunosuppression, immunotoxicity or dysregulation, making workers more susceptible to infections, autoimmune disorders, and chronic inflammation. Refers to the adverse effects of heavy metals on the immune system. Metals like lead and mercury can disrupt normal immune functions, leading to an increased susceptibility to infections, autoimmune diseases, and allergic reactions (Dietert & Piepenbrink, 2006). The mechanisms through which heavy metals exert their toxic effects include the generation of reactive oxygen species (ROS), inhibition of essential enzymatic functions, and interference with cellular signaling pathways. Despite the widespread exposure of workers in the petroleum industry, there is limited comprehensive research that explores the dual impact of heavy metals on both the immunological and hepatological health of petroleum industry employees. This has led to a knowledge gap that hinders effective risk assessment and the development of safety guidelines. Therefore, this manuscript investigates how prolonged exposure to heavy metals impacts the immune and liver health of petroleum industrial workers, with the aim of understanding the mechanisms involved and providing insights for improving workplace safety and health regulations in this high-risk population.

MATERIALS AND METHOD

Study Area

The study areas include; Mbodo Aluu in Ikwerre Local Government Area of Rivers State, Umuahia in Abia State, Nigeria, Choba, in Obio/Akpor Local Government Area of Rivers State. These are major communities in Rivers State and Abia. With Umuahia, in Abia State witnessing little or no oil and gas activities. While Choba and Aluu has major of such activities within Rivers State.

Ethical Considerations

Ethical clearance was obtained from the University of Port Harcourt ethical committee for permission to collect blood samples from subjects for the purpose of this study. Written consent was obtained also from participants and all data were kept anonymous and confidential.

Materials and Equipment

Equipment

Measuring cylinder (Pyrex), Vacutainer bottles, Cover Slip, Centrifuge (Coslab: Cle-110), Pipette Spectrophotometer (Labomed: UVD-3500, USA), Incubator (Shel Lab: SM16, Chempet), Refrigerator (Labtob: LLR-170, Thailand) , Timing device , Vacutainer Needle and Syringe., Test tubes (Pyrex) Reagents All chemicals/reagents and analytical kits used for the analyses were of analytical grade. They include; IgD, IgA, IgM, IgE & IgG standards, Sample diluent, IgD, IgA, IgM, IgE & IgG antibody conjugated to enzyme, Substrate, Stop solution, Microtiter plate coated with anti-human IgD, IgA, IgM, IgE & IgG antibody, Distilled water, Randox kits (Crumlin, Co. Antrim, United Kingdom)

Methods

Sampling Method Blood sample (5ml) was obtained through venipuncture with Vacutainer system and collected into tagged ethylene diamine tetra-acetic acid (EDTA) anti-coagulant and serum- separating tubes. The labeled tubes housing the blood samples were put in cooler boxes lined with ice pack at the point of blood collection. Personal protective equipment (PPE) as required were in use. Heavy Metal Analysis Sample preparation Wet digestion method: a 100ml of HNO_3, H_2SO_4, and HClO in the raito of 40%:40%:20% were mixed together, 1g of sample was measured into conical flask, with 2mL of mixed acid added into each sample in the conical flask. It was thereafter digested/heated in a fume cupboard using hot plate until white fumes were seen. This solution was filtered into a 100ml volumetric flask and made up to mark with distilled water after cooling. Atomic Adsorption Spectrophotometer (AAS) Procedure Heavy metals (Lead, Cadmium, Chromium, Nickel and Zinc) were analyzed using AAS Model: S4=71096, with eight-socket hollow cathode lamps. The gases used in this instrument were acetylene and air. Biochemical Parameters Assay Alanine transaminase (ALT), Plasma Aspartate transaminase (AST), Plasma Alkaline Phosphatase Activity (ALP), and Plasma Gamma glutamyl transferase (GGT) activity were evaluated using the Randox Method while IgG, IgD, IgA, IgM & IgE were determined using Enzyme-Linked Immunosorbent Assay (ELISA).

Statistical Analysis

SPSS software version 23 was used. Mean values (M) ± SEM were calculated and One-Way ANOVA (Analysis Of Variance) test was performed. Significance level was considered at 95 % confidence level ($p<0.05$).

RESULTS

Heavy Metals in Haem from Sampled Individuals

The heavy metal contents of of blood samples obtained from sampled individuals differentially exposure to petroleum products is as shown in table 4.1. The result revealed Pb range from 0.0033±0.001 in control to 0.8908±0.125, Cd 0.00061 in control to 0.019, Cr 0.0044 to 1.982, Ni 0.0016 to 0.218 and Zn 0.1 to 3.18 mg/L. minimum and maximum values for all heavy metals were obtained from Control (Umuahia) and APH(Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups and the control at $p<0.05$.

Table 1: Heavy Metals in Haem from Sampled Individuals

Group	Pb (mg/l)	Cd (mg/l)	Cr (mg/l)	Ni (mg/l)	Zn (mg/l)
Control	0.0033 ± 0.00^{a}	0.00061 ± 0.00^{a}	0.0044 ± 0.0014^{a}	0.0016 ± 0.000^{a}	0.1003 ± 0.04^{a}
MW	0.3958 ± 0.08^{b}	0.0334 ± 0.005^{b}	0.7337 ± 0.0906^{b}	0.2049 ± 0.059^{b}	1.0267 ± 0.17^{b}

MAFL	0.2883±0.10^{b}	0.0151±0.005^{c}	0.9420±0.274^{b}	0.1255±0.044^{b}	1.8838±0.23^{b}
APH	0.8908±0.12^{c}	0.0193±0.004^{c}	1.9820±0.236^{c}	.2182±0.0513^{b}	3.1832±0.47^{c}

Values are Mean±SEM, Means in the same column with same superscript alphabet are not significantly different while means in same column with different superscript alphabets are significantly different at $p \leq 0.05$, n=10

Liver Function Indices in Haem from Sampled Individuals

The Liver function parameters assayed in blood samples obtained from sampled individuals differentially exposure to petroleum products is as shown in table 4.2. The result revealed ALP range from 45.03 to 182.02, ALT 8.00 to 55.70, AST 14.18 to 47.72, GST 5.17 to 15.96 and GGT 11.82 to 49.30 U/L. minimum and maximum values for all hepatological indices were obtained from Control (Umuahia) and APH (Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups and the control at $p<0.05$.

Table 2: Liver Function Indices in Haem from Sampled Individuals

Group	ALP	ALT	AST	GST	GGT
Control	45.03±3.73^{a}	8.00±1.33^{a}	14.18±0.96^{a}	5.17000.55^{a}	11.82±1.47^{a}
MW	175.43±6.06^{b}	55.10±4.17^{b}	41.99±2.42^{b}	13.42±1.31^{b}	44.88±3.43^{b}
MAFL	169.32±4.39^{b}	53.90±3.03^{b}	44.68±1.77^{b}	13.72±1.25^{b}	46.13±2.15^{b}
APH	182.02±20.02^{b}	55.70±6.44^{b}	47.72±2.32^{c}	15.96±1.35^{b}	49.30±3.51^{b}

Values are Mean±SEM, Means in the same column with same superscript alphabet are not significantly different while means in same column with different superscript alphabets are significantly different at $p \leq 0.05$, n=10

Immunological Indices in Haem from Sampled Individuals

The Immunological Indices assayed in blood samples obtained from sampled individuals differentially exposed to petroleum products is as shown in table 4.3. The result revealed IgG range from 10.12 to 29.19, IgE 27.70 to 242.84 (MAFL), IgA 1.63 to 11.68 IgM 1.23 to 9.34 and IgD 34.60 to 259.66. Minimum and maximum values for IgG and IgE were obtained from Control (Umuahia) and MAFL (Filling Station in Aluu) respectively, while minimum and maximum values for IgA, IgM and IgD were obtained from Control (Umuahia) and APH (Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups and the control at $p<0.05$.

Table 3: Immunological Indices in Haem from Sampled Individuals

Group	**IgG**	**IgE**	**IgA**	**IgM**	**IgD**
Control	10.12±0.78^{a}	27.70±8.84^{a}	1.63±0.24^{a}	1.23±0.17^{a}	34.60±4.17^{a}
MW	25.88±1.94^{b}	219.94±15.79^{b}	8.80±0.87^{b}	6.57±0.60^{b}	146.94±10.39^{b}
MAFL	29.10±1.22^{b}	242.84±15.02^{c}	11.41±1.02^{b}	8.91±0.68^{b}	162.54±10.67^{c}
APH	29.16±1.94^{b}	237.08±15.05^{c}	11.68±0.90^{b}	9.34±0.72^{b}	259.66±97.69^{d}

Values are Mean±SEM, Means in the same column with same superscript alphabet are not significantly different while means in same column with different superscript alphabets are significantly different at $p \leq 0.05$, n=10

DISCUSSION

Heavy metals are known as elements with a relatively high density when compared to water (Fergusson, 1990). With the postulation that heaviness and toxicity are inter-alia, metalloids such as arsenic are as well seen as heavy metals, as they are able to induce toxicity even at low concentrations (Duffus, 2002). Recently, environmental and ecological contamination/ pollution by these metals has been of high public health concern globally, as human exposure has risen dramatically due to an exponential rise from several industrial, domestic, agricultural, and technological applications, usages and contamination (Bradl, 2002). International Agency for Research on Cancer (IARC, 1987) classified iron (Fe), zinc (Zn), copper (Cu) and nickel (Ni) amongst others as non-carcinogenic metals, with Pb, As, Cr and Cd being known as both carcinogenic and non-carcinogenic metals known for their widespread role in environmental pollution (Sanborn et al., 2002). Neuropathy, cardiovascular deaths, slow growth development and respiratory illnesses are some prominent diseases associated with exposure to these metals (Turner and Hefzi 2010). The level of these metals obtained from different sample sites as shown in table 1. Revealed notable difference when compared with known standards. The results of the heavy metal contents from blood samples of individuals exposed to various petroleum products pollution sources are shown in table 1. The result revealed Pb range from 0.0033±0.001 in control to 0.8908±0.125, Cd 0.00061 to 0.019, Cr 0.0044 to 1.982, Ni 0.0016 to 0.218 and Zn 0.1 to 3.18 mg/L. minimum and maximum values for all heavy metals were obtained from Control (Umuahia) and APH (Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups and the control at $p<0.05$. The values obtain were above WHO recommended values for all metals except Zn which were quite minimal compared to recommended standards. However, different heavy metals were shown to be spatially different when exposed to various heavy metal pollution sources. The result showed Pb levels were significantly difference between MW and APH, and between MAFL and KPO. Cadmium levels was shown to be significantly different between MW and MAFL and between MW and APH. Chromium blood level was shown to be significantly different between MW and MAFL and between MW and APH. The heme concentration of nickel (Ni), revealed a significantly different level of Ni between MW and MAFL. Zinc (Zn) levels were also shown to be significantly different between MW and MAFL, MW and APH and between MAFL and APH. All the groups were shown to be significantly different when compared to the control at $P<0.05$. Seregin and Ivaniov, (2001) reported that Pb has potentials to alter membrane permeability, water imbalance, inhibits enzyme activities, mineral nutrition disturbance with lots more other lethal effects at high Pb dosage. Report by Uaboi – Egbenni, (2010) also shows Its toxic effect within kidneys, liver etc . Studies revealed that Cadmium even at Low ingestion is toxic and could on chronic exposure lead to; bone disorders, (Asia et al., 2008; Dan-lin et al., 2011). Dayan and Paine, (2001) reported that Cr (III) exposure could be associated with respiratory diseases, with evidenced wheezing, coughing etc. Several reports have shown Pb presence in the even in dust samples, Popoola et al, (2012) reported Pb presence in classroom dusts of schools within Lagos State, Olua et al (2018) also reported presence of heavy metals in dusts samples obtained from classrooms around high anthropogenic crude oil contaminated environment. It has also been reported that Pb may become toxic to plants and animals if their concentrations exceed certain levels (0.0035) as set by USEPA and WHO (Aydinalp and Marinova, 2009), as Pb has the potential to inhibit water imbalance, alter mineral nutrition, enzyme activities, hormonal status and membrane permeability alteration. Reports have shown that Pb at increased concentrations could inhibit cellular activities thus causing cell death (Seregin, and Ivaniov, 2001). It as well has a toxic destructive impact on the kidneys, central nervous system, reproductive system, liver etc with most severe effect being brain necrosis, these hence poses dangerous threat to our environment. (Ademoroti, 1996; Asia et al., 2008; Uaboi – Egbenni, 2010). It's been reported that exposure to Pb can cause damage or reduce children's intelligence and academic performance. It also has potential to decrease hearing ability and children's sight and could cause memory loss and attention deficit disorders. (Sanborn et al., 2002). The mean Cd levels seen is of high public health concern as Gough et al., 1979; Adriano, 1986 and Aydinalp and Marinova, 2009; have independently reported that Cd does not have any known beneficial effects and could become toxic to plants and animals on bioaccummulation whereas Asia et al., 2008 reported that Cd is toxic even if absorption by ingestion is low. Chronic exposure to elevated cadmium levels in food causes bone disorders, including osteoporosis and osteomalacia (Asia et al., 2008). The EPA, (1971) accounted for the moderately toxicity of Cadmium to all organisms, with cumulative poisoning in animals concentrating at the liver, kidney, pancreas and thyroid of humans and other mammals (EPA, 1971). Its major route of entrance in humans is through gastro-intestinal tract by consumption of foods grown on contaminated soil, however smokers may receive a considerable part of their cadmium by inhaling cigarette smoke (Corbett et al., 2002 and Iqbal, 2011). Cadmium levels on the average as observed in this study was above the average concentration in the earth's crust 0.2µg/g (Lewis, 2004), suggesting an anthropogenic Cd source in all sampled sites. Studies have shown that occupational exposure to Cr (III) via inhalation has been associated with respiratory effects with an evidenced wheezing, coughing, etc. (Novey and Habib, 1983). Other observable symptoms includes; hyperemia, asthma, chronic rhinitis, chronic bronchitis, chronic pharyngitis, tracheobronchitis, etc. (Dayan and Paine, 2001). Chromium absorbed via dermal contact could trigger immune response. Sensitized people will showcase allergic dermatitis when exposed to high chromium level (Polak, 2019). Localized vesicular lesions on points of contact or eczematous dermatitis could suggest sensitization (Lewis, 2004; ATSDR, 2020). Lung cancer risk analysis suggests potential excessive death risk from lung cancer amongst U.S. workers exposed to previous permissible limit for Cr (VI) (52 µg m^{-3}) (Braver and Infante, 1985). Recent studies also disclosed excessive mortal lung cancer risk from occupational Cr (VI) compounds' exposure (Gibb et al., 2020; Park et al., 2004). Activities of localized industries within sampled sites might be responsible for the Chromium levels reported in this study as the result showed that Cr is of the highest amount of all heavy metals assayed. The outcome of the plasma hepatospecific enzyme activities in sampled individuals as shown in Table 2 demonstrated significant alterations in activities of these indices as seen in individuals from various sampled locations The result revealed ALP range from 45.03 to 182.02, ALT 8.00 to 55.70, AST 14.18 to 47.72, GST 5.17 to 15.96 and GGT 11.82 to 49.30 U/L. minimum and maximum values for all hepatological indices were obtained from Control (Umuahia) and APH (Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups compared to the control at $p<0.05$. This study corroborates reports that cumulative exposures to crude oil related pollution in laboratory animals resulted in biochemical changes in the liver (Bhawan and Nagar, 2008). High ALT, AST values is suggestive of impairment of liver functions (Tomao et al., 2002). Individuals exposed to petroleum product polluted environments presented highly significant levels of assayed enzymes compared to the control group obtained from Umuahia in Abia State. This is an indication of hepatocellular damage. Elevated levels of liver function markers are often found in blood circulation when the integrity of liver is compromised (Green and Flamm, 2002). The Immunological Indices assayed in blood samples obtained from sampled individuals differentially exposed to petroleum products shown in table 3 also revealed significantly elevated Immunoglobulins. This is suggestive of an immune response to invading or foreign antigens that might have been elicited by the presence of these heavy metals. The result revealed IgG range from 10.12 to 29.19, IgE 27.70 to 242.84, IgA 1.63 to 11.68 IgM 1.23 to 9.34 and IgD 34.60 to 259.66. Minimum and maximum values for IgG and IgE were obtained from Control (Umuahia) and MAFL (Filling Station in Aluu) respectively, while minimum and maximum values for IgA, IgM and IgD were obtained from Control (Umuahia) and APH (Artisan petroleum handlers in Mgbodo Aluu) respectively. The obtained results showed significant difference between the various groups and the control at $p<0.05$. Immunoglobulins (Igs) are produced by B cells and contribute to the immune response via antigen binding and/or by mediating specific effector functions (Schroeder, 2010; Kaneko et al., 2000 and Griffin et al., (2000)). Both IgG, the most abundant Ig in the body, and IgM, the initial Ig expressed in response to an acute infection, play critical roles in neutralizing toxins and other immunogens, whereas increased production of IgE specifically is closely associated with hypersensitivity and allergic responses (Kaneko et al., 2000). Hence, values obtained from this study is suggestive of hypersensitivity and allergic responses and is also indicative that the body treats these heavy metals as foreign bodies and or toxins. The results revealed significant difference between the various groups and the control at $p<0.05$ degree of confidence. The IgA levels were shown to be significantly different between MW and MAFL, and between MW and APH. While IgM were significantly different between MW and MAFL, and between MW and APH.The findings suggest that individuals exposed to petroleum products, particularly artisan petroleum handlers, have significantly higher levels of heavy metals in their blood, accompanied by elevated liver function markers and immunological indices. This indicates both hepatotoxic and immunotoxic effects, with a strong association between exposure to heavy metals and adverse health outcomes in these populations. The significant differences observed between the exposed groups and the control emphasize the health risks associated with occupational and environmental exposure to petroleum products. This observation is consistent with the findings of Johnson et al. (2020), who reported that chronic exposure to lead resulted not only in immune system activation but also in subsequent immunosuppression, characterized by reduced T-cell counts and impaired immune responses.

CONCLUSION

The elevated levels of heavy metals, liver enzymes, and immunological parameters observed in this study suggest significant hepatotoxic and immunotoxic effects. These findings contribute to the growing body of evidence linking heavy metal exposure to serious health risks and underscore the need for preventive measures to protect vulnerable populations.

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