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Cadmium and Lead Induced Proinflammatory Cytokine Polarization in Petroleum Products Occupationally Exposed Nigerians

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Abstract

The toxicological effects of petroleum product had been associated with various metabolic defects such as cardio-vascular attacks. But there is limited information about the cytokine responses of individual who are occupationally exposed to petroleum products in Nigeria. The study was aimed at evaluating the cytokine responses to the accumulation of cadmium, lead, zinc and copper in the petroleum products using blood levels of interleukin-1 alpha, interleukin-4, cadmium, lead, zinc and copper in occupationally exposed individuals in Abuja, Nigeria. The case-control study randomly selected sixty-three occupationally exposed (test group) and sixty non exposed individuals (control group). The occupationally exposed subjects, consisting of twenty-four automobile mechanics (AM), eighteen generator mechanics (GM) and twenty-one petrol station attendants (PSA) in filling stations who consented to participate in this study. Cytokine levels were determined by enzyme-linked immunosorbent assay method. Heavy metals were analyzed by atomic absorption spectrophotometer method. Full blood count was done using hematology auto-analyzer. All numerical results were analyzed with one-way ANOVA with post hoc multiple comparisons test while student's t- test was used to compare independent variables. Cadmium levels were significantly higher in AM and PSA ($p<0.002$) while lead levels were significantly higher in AM and GM ($p<0.04$) as compared with the control group. Zinc and copper levels were significantly lower in AM and GM as compared with the control group ($P<0.002$) but within the acceptable levels. Granulocytes count was significantly higher in GM and PSA, whereas, red blood cells count and packed cell volume were significantly lower in GM and PA as compared with control group ($p<0.001$). IL-1α and IL-4 levels were significantly higher in AM, GM and PSA as compared with the control group ($p<0.01$; 0.017) respectively. Regardless of profession, prolonged exposure to petroleum products tends to shift the cytokine balance toward a pro-inflammatory pattern. Thus, when handling such materials, personal protective equipment should be worn to avoid the accumulation of cadmium and lead, which might act as mediators.

Keywords: Cytokine responses • Cadmium • Lead • Antioxidant trace • Ooccupationally exposed • Cytokine balance

Introduction

Study area

This study was carried out in Nyanya, Abuja Municipal Area Council (AMAC), which is one of the six area councils of the Federal Capital Territory, Abuja, Nigeria. The case-control study randomly selected sixty-three occupationally exposed (test group) and sixty (60) non exposed individuals (control group). The occupationally exposed individuals, consisting of twenty-four automobile mechanics, eighteen generator mechanics and twenty-one petrol station attendants in filling stations who consented to participate in this study. These occupations are among those likely to expose individuals to the heavy metals studied. The sixty controls were apparently healthy individuals of Nyanya, Abuja who were not occupationally exposed to petroleum products. The length of exposure was captured by administering a self-developed semi-structured questionnaire.

Inclusion criteria: All the subjects were within the age group of eighteen to fifty years. The subjects were actively serving as petrol station attendants, automobile mechanics or generator mechanics with a minimum of one year exposure.

Exclusion criteria: Individuals who are not presently serving as petrol station attendants, auto mechanics or generator mechanics were excluded from the occupationally exposed group. Also excluded from the occupationally exposed groups were those residing in and around petrol stations and traffic congestion area.

Informed consent and ethical approval: Informed consent was obtained from all subjects for this study and ethical approval obtained from the Health Research Ethics Committee of the Directorate of Health and Human Services, Federal Capital Territory, Abuja.

Collection of sample: For the heavy metals determination, about 3 ml of venous blood samples were collected aseptically into Potassium ethylene diamine tetra acetic acid (K-EDTA) anticoagulant tubes. The samples were labelled and immediately placed in ice pack at the site of collection and subsequently transferred into a refrigerator at 4°C. For the full blood count (FBC) and Interleukin assays, 4 ml of venous blood were collected into

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K-EDTA tubes, mixed and FBC carried out immediately. The blood samples were centrifuged within 30 minutes of collection for 15 minutes at 2000 rounds per minute (RPM) and plasma separated from the cells into plain tubes and refrigerated at 2-8ºC [1-7].

Determination of blood cadmium, lead, zinc, copper levels and cytokines: A calibrated Buck 205 flame atomic absorption spectrophotometer (Perkin-Elmer, HGA-2100) was used to analyze the blood sample for Cd, Pb, Zn and Cu as described by Inyengar et al. (7) at Yitzhak Rabin Laboratory for Advanced Micropropagation and Biotechnology Research Centre, Nnamdi Azikiwe University Awka, Nigeria. This method is based on the principle that atoms of the element when aspirated into Atomic Absorption Spectrophotometer vaporizes and absorbed light of the same wavelength as that emitted by the element when in the excited state. The amount of light absorbed can be correlated in a linear fashion to the concentration of the analyte in the sample. The levels of IL-1βand IL-4 were determined using enzyme-linked immunosorbent assay (ELISA) method as described by Alfred E F, et al. [8].

Statistical analysis: All numerical results were analyzed with one-way ANOVA with post hoc multiple comparisons test while student's t- test was used to compare independent variables.

Results

(Table 1) shows the demographic characteristics of the subjects. It shows the total number of subjects that participated in the study per occupation. It also shows the mean levels of ages (years) of the subjects in the different occupational groups. It also showed the mean exposure (years) of the subjects in the individual occupations. (Table 2) shows the mean ± (SD) levels of heavy metals in the different occupational groups as compared with the control group. The levels of cadmium were significantly higher in auto mechanics and petrol station attendants as compared with the control group (p<0.002). The mean levels of Lead were significantly higher in auto mechanics and generator mechanics (0.04), while the level of zinc and copper were significantly lower in auto mechanics and generator mechanics as compared with the control group (P<0.002) but within the acceptable levels. (Table 3) shows the mean ± (SD) levels of haematological indices of the subjects in different occupational groups as compared with the control group. The levels of total white blood cells were significantly higher in auto-mechanics and generator mechanics as compared with the control group (p<0.001). The levels of granulocytes was significantly higher in generator mechanics and petrol station attendants compared with the control group (p<0.001), while the levels of red blood cells count and packed cell volume were significantly lower in generator mechanics and petrol station attendants as compared with control group (p<0.001). The mean levels of MCH were significantly higher in generator mechanics when compared with the control group (P<0.003). (Table 4) shows the mean ± (SD) levels of cytokines in different occupational groups compared with the control group. The levels of IL-1βand IL-4 were significantly higher in auto mechanics, generator mechanics and petrol station attendants as compared with the control group (p<0.01; 0.017) respectively.

Discussion

Depending on the degree and length of exposure, cadmium and lead exposure can have a variety of biological consequences, including toxic effects on the haematological, cardiovascular, neurological, and reproductive systems [9-14]. According to the findings of this study, cadmium levels are significantly higher in auto mechanics and gas station attendants, whereas lead levels are significantly higher in car mechanics and generator mechanics. This could be related to the group's heavy lubricant usage, which exposed them to high levels of cadmium and lead. Lead and cadmium exposure may also come through practices like hand cleaning with petroleum products and sucking. Fenga C, et al. [15] and Babalola OO, et al. [16] previously found elevated levels of lead among petroleum product occupationally exposed individuals, which were linked to the continued use of leaded gasoline for automobiles in Nigeria. However, prolonged exposure to these petroleum products as seen in (table 1). could also be a contributing factor to the accumulation of cadmium and lead to these group of individual. It had also been observed that prolonged exposure to petroleum compounds *via* ingestion, gastrointestinal tract, and inhalation from burning fossil fuel might raise heavy metal levels in exposed patients such as cadmium and lead [17]. A study by Musa AM, et al.,

Table 1. Statistical analysis of age and length exposure values of the studied subjects (mean ± SD).

Parameters	Automobile mechanics (n=24)	Generator mechanics (n= 18)	Petrol attendants (n =21)	Control (n = 60)
Age (years)	26.9 ± 3.17	35.5 ± 2.08	34.4 ± 3.02	31.9 ± 2.02
Length of exposure (years)	5.6 ± 1.59*	9.7 ± 2.79*	9.1 ± 2.25*	0.0 ± 0.00

*Significant levels were considered at p< 0.05

Table 2. Statistical analysis of heavy metals values in the three occupationally exposed subjects (mean ± SD).

Parameters	Automobile mechanics	Generator mechanics	Petrol attendants	Control	F-value	P-values
Cadmium (µg/L)	1.25 ± 0.06*	1.11 ± 0.09	0.24 ± 0.10*	0.05 ± 0.01	6.308	0.002
Lead (µg/L)	2.42 ± 0.58*	3.97 ± 0.23*	2.22 ± 0.13	0.13 ± 0.32	3.175	0.04
Zinc (µg/L)	163 ± 0.6.7*	159 ± 0.8.3*	156 ± 0.08*	221 ± 0.54	7.176	0.002
Copper (µg/L)	1.7 ± 0.08*	1.7 ± 0.07*	2.0 ± 0.04	2.2 ± 0.05	3.614	0.024

*Significant levels were considered at p< 0.05

Table 3. Statistical analysis of Haematological indices in the three occupationally exposed subjects (mean ± SD).

Parameters	Automobile mechanics	Generator mechanics	Petrol attendants	Control	F-value	P-values
WBC (10^3)	6.7 ± 0.44*	7.0 ± 0.62*	5.3 ± 0.39	4.9 ± 0.24	7.301	0.001
LYM (%)	48.3 ± 2.75	42.2 ± 4.01	47.8 ± 2.51	36.8 ± 2.89	3.644	0.025
Gran (%)	49.1 ± 2.33	63.8 ± 2.83*	60.6 ± 2.73*	46.5 ± 1.64	13.73	0.001
Mid (%)	3.7 ± 0.36	3.6 ± 0.26	3.5 ± 0.26	3.9 ± 0.35	0.324	0.808
RBC (10^6)	5.8 ± 0.23	4.8 ± 0.15*	4.6 ± 0.19*	5.5 ± 0.14	8.807	0.001
HCT (%)	45.8 ± 1.36	42.3 ± 1.57	37.8 ± 1.62*	45.0 ± 0.66	8.662	0.001
MCV (ft)	82.6 ± 1.94	87.7 ± 1.45	82.6 ± 1.82	85.0 ± 0.98	2.065	0.125
MCH (pg)	27.3 ± 0.72	30.2 ± 0.52*	27.5 ± 0.76	26.1 ± 0.57	5.650	0.003
MCHC (g/dL)	33.0 ± 0.14	33.5 ± 0.33	33.2 ± 0.29	33.2 ± 0.14	0.787	0.509

*Significant levels were considered at p< 0.05

Table 4. Statistical analysis of cytokines values in the three occupationally exposed subjects (mean ± SD).

Parameters	Automobile mechanics	Generator mechanics	Petrol attendants	Control	F-value	P-values
Interleukin 1α (ng/L)	336.9 ± 0.02*	361.8 ± 0.05*	326.1±0.08*	16.5 ± 0.05	3.414	0.001
Interleukin 4 (ng/L)	12.1 ± 0.05*	12.0 ± 0.04*	10.2 ± 0.06*	17.4 ± 0.07	3.944	0.017

*Significant levels were considered at p< 0.05

[18], observed a lower cadmium level in the same group of subjects in Zaria, Nigeria but these group of individuals where acutely exposed to petroleum products, while the level of zinc and copper were significantly lower in auto mechanics and generator mechanics. This observation could be linked to cadmium and lead induced competitive inhibition of metalloenzyme on the site of absorption of zinc and copper which resulted to poor absorption of zinc and copper from the gastrointestinal tract. A study carried out by Flora SJS, et al., [19], showed that shows that high levels of calcium can competitively inhibit the absorption of zinc and lead from the gastrointestinal tract. Studies in both animals and humans have shown that a deficiency in essential metals such as zinc [20], calcium [21] or iron [22] can lead to greater absorption and toxicity of Cd and Pb. It had earlier been reported that zinc has similar chemical and physical properties to Cd and Pb, it competes for the binding sites of metal absorptive and enzymatic proteins [21]. Therefore it could be postulated that the supplementation with zinc could have provide protective effects against Cd and Pb accumulation but these is not within the scope of this present study but studies have shown that appropriate concentrations of essential metal supplementation are helpful in preventing complications associated with Cd and Pb accumulations [23,24].

Furthermore, the observed increased in the granulocytes count and decrease in red blood count and packed cell volume in the generator mechanics and petrol station attendant's couples with the significantly increased levels of IL-1βand IL-4 across the three different occupational exposed groups (Tables 2-4), could be linked to Cd-Pb induced cytokines dysfunction caused by the accumulation of Cd and Pb in the peripheral blood. It had been reported that Cd and Pb exposure cause the loss of essential metals which are needed to prevent oxidative stress as seen in cases of iron-deficiency anaemia and osteoporosis [25,26]. However, it obvious from these study that the accumulation of Cd and Pb acts as an immunotoxic agent by selectively up-regulating the pro-inflammatory cytokines while down-regulating the anti-inflammatory cytokines. Studies have shown that the accumulation of heavy metals such as cd and Pb promotes the expression of the pro-inflammatory factors IL-1b, IL-6, and TNF-βin immunological cell by increasing ROS production, while inhibiting that of the anti-inflammatory cytokine IL-10 [27-30]. Cao X, et al. [31] showed that Cd accumulation can activates the mitochondria-mediated internal apoptosis pathway in BEAS2B cells, thus reducing their viability, causing reactive oxygen species (ROS) accumulation, inducing apoptosis, suppressing Bcell lymphoma-2 expression, and enhancing B-cell lymphoma-2- associated X and cleaved caspase-3 protein expression. Pathak N and Khandelwal S [29] showed that Cd-treated splenocytes and thymocytes produce large amounts of ROS, which not only serve as a key mediator of Cd-induced apoptosis/ cellular damage. Furthermore, Cd exposure inhibited the activity of chicken peritoneal macrophages and promoted the expression of IL-1b, IL-6, and TNF-a in both inactivated macrophages and cells in response to LPS stimuli Yucesoy B, et al. [27]. Thus, it is possible that Cd and Pd accumulation can cause cytokine dysfunction and then activate abnormal cytokine signaling pathways to produce the abnormal cytokine responses observed in this study. A better understanding of the pathways of Cd and Pb induced cytokines dysfunction, as well as interventions using inhibitors such as Zn and Cu, should be developed to effectively reduce Cd and Pb induced cytokines dysfunction seen in individuals exposed to petroleum products on the job in Nigeria.

Conclusion

Regardless of profession, prolonged exposure to petroleum products tends to shift the cytokine balance toward a pro-inflammatory pattern. Thus, when handling such materials, personal protective equipment should be worn to avoid the accumulation of cadmium and lead, which might act as mediators.

Competing Interests

The authors declare that they have no competing interests.

Authors Contributions

This work was carried out in collaboration between all authors. Author EFA and MMD designed the study and performed the statistical analysis. Authors EFA, MMD, DAK, UNE, EJI, EAO, URE and EPI conducted and managed the Laboratory analysis. All authors read and approved the final manuscript.

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References

1. Robertson, Graham, Isabelle Leclercq and Geoffrey C. Farrell. "II. Cytochrome P-450 enzymes and oxidative stress." *Am J Physiol Gastrointest Liver Physiol* 281 (2001): G1135-G1139.
2. Hsiao, Chu-Lin, Kuan-Hsun Wu and Kong-Sang Wan. "Effects of environmental lead exposure on T-helper cell-specific cytokines in children." *J Immunotoxicol* 8 (2011): 284-287.
3. Galadima, A and Z. N. Garba. "Heavy metals pollution in Nigeria: causes and consequences." *Elixir pollution* 45 (2012): 7917-7922.
4. Dobrakowski, Michał, Marta Boroń, Zenon P. Czuba and Ewa Birkner, etal. "Blood morphology and the levels of selected cytokines related to hematopoiesis in occupational short-term exposure to lead." *Toxicol Appl Pharmacol* 305 (2016): 111-117.
5. Herman, D'souza Sunil, Menezes Geraldine, Clark C. Scott and Thuppil Venkatesh. "Health hazards by lead exposure: Evaluation using ASV and XRF." *Toxicol Ind Health* 22 (2006): 249-254.
6. Han, Jiahuai and Richard J. Ulevitch. "Limiting inflammatory responses during activation of innate immunity." *Nat Immunol* 6 (2005): 1198-1205.
7. Iyengar, G., Subramanian, K and Woittiez, J. Principles and practice boca raton: CRC pres sample decomposition in: Element Analysis of biological samples 6 (2012) 103–125.
8. Alfred, E. F., A. Benson Olu, E. Imuetiyan Joy and J. Sunday, et al. "The levels of c-reactive protein, malondialdehyde and absolute lymphocyte counts in pre and post-acute exercise." *J Sports Med Doping Stud* 7 (2017): 2161-0673.
9. Tang, Ning and Zi Q. Zhu. "Adverse reproductive effects in female workers of lead battery plants." *Int J Occup Med Environ Health* 16 (2003): 359-361.
10. Navas-Acien, Ana, Eliseo Guallar, Ellen K. Silbergeld and Stephen J. Rothenberg. "Lead exposure and cardiovascular disease - A systematic review." *Environ Health Perspect* 115 (2007): 472-482.
11. Sanders, Talia, Yiming Liu, Virginia Buchner and Paul B. Tchounwou. "Neurotoxic effects and biomarkers of lead exposure: A review." *Rev Environ Health* 24 (2009): 15-46.
12. Nigra, Anne E., Adrian Ruiz-Hernandez, Josep Redon and Maria Tellez-Plaza. "Environmental metals and cardiovascular disease in adults: A systematic review beyond lead and cadmium." *Curr Environ Health Rep* 3 (2016): 416-433.
13. Bertke, Stephen J., Everett J. Lehman, Steven J. Wurzelbacher and Misty J. Hein. "Mortality of lead smelter workers: A follow-up study with exposure assessment." *Am J Ind Med* 59 (2016): 979-986.

14. Rzymski, Piotr, Katarzyna Tomczyk, Barbara Poniedzialek and Maciej Wilczak. "Impact of heavy metals on the female reproductive system." *Ann Agric Environ Med* 22 (2015).
15. Fenga, C., C. Catania, C. Di Nola and A. Cacciola, et al. "Air pollution in urban areas as a risk factor for health in the general population and in workers. Environmental and biologic monitoring of 1-3 butadiene." Giornale Italiano di Medicina del Lavoro ed Ergonomia 29 Suppl (2007): 861-863.
16. Babalola, O. O., L. O. Ojo and M. O. Aderemi. "Lead levels in some biological samples of auto-mechanics in Abeokuta, Nigeria." (2005).
17. Usuda, Kan, Koichi Kono, Keiko Ohnishi and Shin Nakayama, etal. "Toxicological aspects of cadmium and occupational health activities to prevent workplace exposure in Japan: A narrative review." *Toxicol Ind Health* 27(2011): 225-233.
18. Musa, A., M. Garba, I. A. Yakasai and M. T. Odunola. "Determination of blood levels of cadmium and Zinc in humans from Zaria, Nigeria." New Clues in Sciences 2 (2011): 49-54.
19. Flora, S. J. S., M. Mittal and A. Mehta. "Heavy metal induced oxidative stress & its possible reversal by chelation therapy." *Altern Med* 14 (2009): 87-88.
20. Reeves, Philip G and Rufus L. Chaney. "Marginal nutritional status of zinc, iron and calcium increases cadmium retention in the duodenum and other organs of rats fed rice-based diets." *Environ Res* 96, (2004): 311-322.
21. Bridges, Christy C and Rudolfs K. Zalups. "Molecular and ionic mimicry and the transport of toxic metals." *Toxicol Appl Pharmacol* 204 (2005): 274-308.
22. Hammad, Tarek A., Mary Sexton and Patricia Langenberg. "Relationship between blood lead and dietary iron intake in preschool children: A cross-sectional study." *Ann Epidemiol* 6 (1996): 30-33.
23. Amara, Salem, Hafedh Abdelmelek, Catherine Garrela and Pascale Guiraud, et al. "Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis." *J Reprod Dev* 54 (2008): 129-134.
24. Prasanthi, RP Jaya, C. Bhuvaneswari Devi, D. Chand Basha and N. Saya Reddy, etal. "Calcium and zinc supplementation protects lead (Pb)-induced perturbations in antioxidant enzymes and lipid peroxidation in developing mouse brain." *Int J Dev Neurosci* 28 (2010): 161-167.
25. Jain, Nitin B., Francine Laden, Ulrich Guller and Anoop Shankar, et al. "Relation between blood lead levels and childhood anemia in India." *Am J Epidemiol* 161 (2005): 968-973.
26. Schutte, Rudolph, Tim S. Nawrot, Tom Richart and Lutgarde Thijs, et al. "Bone resorption and environmental exposure to cadmium in women: A population study." *Environ Health Perspect* 116 (2008): 777-783.
27. Yucesoy, B., A. Turhan, M. Üre and T. Imir et al. "Effects of occupational lead and cadmium exposure on some immunoregulatory cytokine levels in man." *Toxicology* 123 (1997): 143-147.
28. Hemdan, Nasr YA, Frank Emmrich, Khadiga Adham and Gunnar Wichmann, et al. "Dose-Dependent modulation of the *in vitro* cytokine production of human immune competent cells by lead salts." *Toxicological Sci* 86 (2005): 75-83.
29. Pathak, Neelima and Shashi Khandelwal. "Impact of cadmium in T lymphocyte subsets and cytokine expression: Differential regulation by oxidative stress and apoptosis." *Biometals* 21 (2008): 179-187.
30. Zhang, Ding, Xiao-yu Yang, Ying-ze Qin and Guo-dong Wu, et al. "Antagonistic effect of N-acetyl-L-cysteine against cadmium-induced cytotoxicity and abnormal immune response on chicken peritoneal macrophages." *Ecotoxicol Environ Saf* 206 (2020): 111185.
31. Cao, Xiangyu, Mingyang Fu, Ruochen Bi and Xiaomei Zheng, et al. "Cadmium induced BEAS-2B cells apoptosis and mitochondria damage *via* MAPK signaling pathway." *Chemosphere* 263 (2021): 128346.

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