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LIPID PEROXIDATION DAMAGE CAUSED BY HEAVY METAL EXPOSURE IN RESIDENTS OF AN OIL PRODUCING AREA IN BAYELSA, STATE, NIGERIA

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ABSTRACT
Blood samples were collected from 200 residents each from Igbeta-Ewoama (Oil Producing Area) and Odi (Non-Oil Producing Area) both in Bayelsa State, Nigeria, to determine the levels of Cadmium, Chromium, Lead, Mercury, Selenium and 8-Iso-Prostagladin $F_2\alpha$. Blood samples were also analyzed for hemoglobin, platelets, white blood cells, prothrombin time and serum concentrations of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, albumin, bilirubin, gamma glutamyl transferase, total protein, sodium, potassium, chloride, bicarbonate, creatinine and urea. Residents in Igbeta-Ewoama had the highest blood levels of 8-Iso-Prostagladin $F_2\alpha$, Mercury, Lead, Selenium, Cadmium and Chromium. These concentrations were all significantly different from levels measured in the non-oil producing community at $P<0.05$. Residents in Igbeta-Ewoama also had the highest mean values of all the measured liver and kidney functions parameters with the exception of albumin, total protein, sodium, chloride and bicarbonate. These parameters were all statistically different at $P<0.05$. There were significant differences in the levels of hemoglobin, white blood cells and platelets, while the length of bleeding time was prolonged in the residents of Igbeta-Ewoama community. The findings of this study suggest that the probability of occurrence of diseases associated with metal toxicity and oxidative stress induced by lipid peroxidation damage might be higher among people living in the oil producing area.

KEYWORDS: Heavy Metal, Lipid Peroxidation, Oil Producing Area, Spillage, Bioaccumulation and Bayelsa State.

INTRODUCTION
Pollution caused by oil production activities is a common phenomenon in Bayelsa state and other states within the Niger Delta region of southern Nigeria where crude oil is found. This is because different amounts of petroleum products and its wastes are discharged into the surrounding environments in the course of these activities causing serious environmental, social, economic and above all health problems. Crude oil contains different proportions of heavy metals namely Zinc (Zn), Lead (Pb) Manganese (Mn), Chromium (Cr), Cadmium (Cd), Iron (Fe), Nickel (Ni), Cobalt (Co), Vanadium (Vd), Mercury Hg), Copper (Cu), Molybdenum (Mo) and Selenium (Se). Nigeria crude oils have relatively high concentrations of Fe, Zn, Cu, Pb and Hg.[1]

Heavy metals represent a unique aspect of toxicology as they do not undergo breakdown into other metals. Unlike organic contaminants, they are not degraded further and also cannot decompose into other chemicals with time.[2] This results to their bioaccumulation in the ecosystem, agriculture and the human body. Heavy metals exhibit toxicity at low concentrations and the toxic effects can last for very long periods due to their accumulation in the biota. A peculiar feature exhibited by these metals is the induction of oxidative stress.[3] Electrons escape from the metals and interact with oxygen to generate reactive oxygen species (ROS) such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical (•OH), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2).[4] Each of these ROS is highly reactive and unstable due to the fact that they contain an unpaired electron in their outer electron shell. Oxidative Stress is a condition characterized by an imbalance between the concentration of Reactive Oxygen Species (ROS) and antioxidants in a living system. Excessive accumulation of ROS will lead to cell injury due to damage to nuclear materials (DNA/RNA), proteins, lipid membranes or by oxidatively inactivating specific enzymes by oxidation of Co-factors.[5]

Oxidative stress is now recognized to be a prominent feature of many acute and chronic diseases in humans. These include cancer, cardiovascular disease, neurodegenerative disease, lung disease and even the normal aging process.[6] However, definitive evidence for this association had been lacking due to recognized shortcomings with methods available to assess oxidative stress status *in vivo* in humans.[7] Several in vitro markers of oxidative stress are available, but most are of limited value *in vivo* because they lack sensitivity and/or specificity or may even require invasive procedures for their analysis.[8] Isoprostanes are prostaglandin (PG) -like substances that are produced in vivo independently of cyclooxygenase (COX) enzymes, primarily by free radical-induced peroxidation of arachidonic acid.[9] The formation of PG-like compounds during auto-oxidation of polyunsaturated fatty acids was first reported in the mid-1970s[10], but isoprostanes were not discovered to be formed in vivo in humans until 1990.[9] Following the discovery of isoprostanes, it has now been established that the measurement of F2-isoprostanes is the most reliable approach to assess oxidative stress status *in vivo* thus, providing an important tool to explore the role of oxidative stress in the pathogenesis of human disease. In addition, products of the isoprostane pathway have been found to exert potent biological actions and therefore may be patho-physiologic mediators of diseases.[11]

Availability of metal ions to biological processes is often dependent on solubility. Soluble salts of metals readily dissociate in the aqueous environment of biological membranes, making transport into the body easy. On the other hand, insoluble salts are poorly absorbed (for example, reduction of chromium (VI) to the less soluble chromium (III) will decrease its absorption).[12] Absorption of solute salts may be modified by the formation of insoluble compounds in biological materials. A typical example is the reduction of lead absorption by high dietary levels of phosphate due to the formation of insoluble lead phosphate. Some metals are produced as alkyl compounds. These are often very lipid soluble and readily pass across the lipid phase of biological membranes. Examples include methylmercury and organo-tin compounds. Strong attractions between metal ions and organic compounds will influence the deposition of metals and their rate of excretion. Most of the toxicologically important metals bind strongly to tissues, and are only excreted slowly and therefore tend to accumulate on continued exposure. Affinities for different tissues vary significantly. Elements such as lead are bound in the bones while mercury and cadmium localize in the kidneys.[13] Though the bulk of Nigeria's revenue is derived from crude oil activities in the Niger Delta region, considerations are rarely made on how oil exploration processes create environmental, health, and social problems in local communities near oil producing fields. The necessity for this study is based on the fact that there is bioaccumulation of heavy metals in plants growing in crude oil contaminated soil.[14] These plants include food crops such as yam, cassava, vegetables and fruits. These plants are consumed by both man and animals living in these areas. As part of the food chain, the animals that feed on the plants are equally consumed by residents in these areas, thus doubling the harmful effects. Some of these plants are equally used for medicinal preparations and thus in the bid to find curative solutions for medical problems, harmful agents may be inadvertently introduced into the body system. Heavy metals are equally bio-accumulated in aquatic species/animals living in crude oil polluted environments.[15] These aquatic species include fishes, crustaceans (crabs, crayfish, lobsters, shrimps prawns etc) and molluscs (periwinkles and snails). These aquatic species are widely consumed in these areas as they serve as the major protein source for the residents. They are equally sold in different markets to unsuspecting consumers. There are high levels of heavy metals in crude oil impacted rivers above the WHO acceptable limits.[16] These rivers in most cases serve as the only source of water for drinking, cooking and all that water is needed for to the residents in these areas.

From the above stated facts, it becomes expedient to assess the levels of heavy metals and oxidative stress markers in residents of these communities who are exposed to these pollutants, drink from the polluted water, eat plants harvested from the surrounding soil and equally consume aquatic species found in these environments. Bearing in mind the cumulative health effects that may result from these, this study tried to explore the impact of crude oil contamination on human health to improve mitigation efforts and also to prevent adverse health effects on individuals living in affected areas.

MATERIALS AND METHODS

Study Area

This study was done in two communities in Bayelsa state, Nigeria.

(i) Igbeta-Ewoama (Oil Producing Area). Igbeta-Ewoama is a community in Nembe Local Government Area of Bayelsa State. Several oil wells operated by the Nigeria Agip Oil Company (NAOC) are found in this area. Oil drilling activities in this place has been on for over 40 years. Inhabitants in this area are mainly fishermen. Many aquatic species such as fishes, lobsters, shrimps, crabs oysters, periwinkles and other crustaceans are abundantly found in this area.

(ii) Odi (Non-Oil Producing Community).The community is located in Kolokuma/Opokuma Local Government Area of Bayelsa state.

These two communities are located in Bayelsa State which lies within, latitude 04^0 15^1 North, 05^0 23^1 South and longitude 05^0 22^1 West and 06^0 45^1 East.[17] Igbeta-Ewoama is the test community while Odi community was used as control.

Study Population
A total number of 400 subjects were recruited for this study. This comprised:
a. 200 residents of Igbeta-Ewoama community (Oil Producing Area)
b. 200 residents from Odi (Non-oil producing community) as control.

Out of the 200 subjects from Igbeta-Ewoama community, 77 were males between the ages of 2 and 77 years. 123 were females between the ages of 2 and 80 years. In Odi community, 106 were males aged between 2 and 80 years while 94 were females aged between 2 and 79 years.

Advocacy and Mobilization
Bayelsa State Ministry of Health granted ethical clearance for this work. The traditional rulers and leaders of the community development committees of each community were met and informed about the study that was to be carried out in their various communities. Their co-operation and support were solicited in mobilizing their subjects. Meetings were held with the members of the communities and informed consent was obtained from the subjects recruited into the study.

Selection Criteria
Questionnaire was used to obtain the required information needed for including or excluding participants.

Inclusion Criteria
Subjects two years and above that consented to the study were included. The sample population was classified according to sex and age groups.

Exclusion Criteria
Subjects with known illnesses such as cancer, diabetes mellitus and Parkinson's disease as well as tobacco smokers were excluded from this study. This is because cigarette smoke is an exogenous source of oxidative stress[18] while levels of oxidative stress biomarkers are known to be raised in the above mentioned disease conditions.[19]

Sample Collection
Blood samples were collected by venipuncture using pyrogen free sterile disposable syringes. Samples for measurement of 8-Iso-$PGF_2\alpha$ and biochemical parameters were collected into plain serum separating tubes. These were allowed to stand for 10-20 minutes after which they were centrifuged at 3,000 rpm for 20 minutes and the serum separated using a Pasteur's pipette. Samples for measurement of hematological parameters (prothrombin time not inclusive) and heavy metals were collected into K_3 EDTA anti coagulated plastic bottles and mixed thoroughly by gentle repeated turning. Samples for prothrombin time were dispensed into containers containing 3.2% tri sodium citrate at a ratio of 9 parts of blood to 1 part of 3.2% tri sodium citrate. The samples were centrifuged for 15 minutes at 3000 rpm to obtain platelet poor plasma. The supernatant plasma was subsequently transferred into plain appendorf tubes.

Laboratory Procedures
All the reagents used for this study were commercially purchased from Afro Famous Nigeria Limited, Abakpa Nike, Enugu, Enugu State and all manufacturers' standard operating procedures (SOPs) were followed strictly.

(A) 8-Isoprostaglandin F_2 α (8-Iso-$PGF_2\alpha$).[20]
The ELABSCIENCE 8-Iso-$PGF_2\alpha$ competitive ELISA technique kit was used for the quantitative measurement of 8-Iso-$PGF_2\alpha$ in the subjects.
(B) Measurement of Cd, Se, Hg, Pb and Cr
Measurement of these metals was carried out on 240 FS AA Agilent Technologies flame atomic absorption spectrometer with deuterium lamp background correction.
(C) Alanine Aminotransferase (ALT)[21]
RANDOX ALT kit was used.
(D) Aspartate Aminotransferase (AST)[21]
RANDOX AST test kit was used.
(E) Albumin[22]
RANDOX Albumin test kit was used.
(F) Bilirubin[23]
RANDOX Bilirubin kit was used.
(G) Alkaline Phosphatase (ALP).[24]
TECO Diagnostics, California, USA direct colorimetric ALP reagent kit was used.
(H) Gamma-glutamyl Transferase (γ-GT)[25]
RANDOX colorimetric (Kinetic Method) test kit was used.
(I) Urea[26]
RANDOX Urease-Berthelot Colorimetric method kit was used.
(J) Creatinine[27]
RANDOX Creatinine kit was used.
(K) Electrolytes (Na^+, K^+, Cl^-, HCO_3^-)[28]
EA-1000B ISE electrolyte analyzer from Perlong Medical Equipment Company was used to measure these parameters.
(L) Platelets, Haemoglobin (Hb).[29]
SYSMEX pocH-100i automated haematology analyzer was used for the measurement of platelets in the study population.
(M) Prothrombin Time (PT)[30]
AGAPE Diagnostics Switzerland Prothrombin Time kit was used.

Statistical Analysis
Data obtained was analyzed using Statistical Package for Social Sciences (SPSS) statistical software (Version 17 for windows) (SPSS Inc, Chicago, USA). Results were expressed as mean and standard deviation and were presented in tables. Test of significance was done using Z- test, Pearson correlation coefficient statistics and

Tukey HSD post HOC test. Values above 95% confidence limit were considered statistically significant.

RESULTS

Table 1 shows blood heavy metal and serum 8-Iso-$PGF_2\alpha$ concentrations measured in the two study groups. Significantly higher levels of all the measured heavy metals and 8-Iso-$PGF_2\alpha$ were observed in subjects residing in Igbeta-Ewoama community ($p < 0.05$).

Table 1: Heavy Metal and 8-Iso-$PGF_2\alpha$ Levels in the Study Populations.

Study Community	Cd(ppm)	Cr(ppm)	Hg(ppm)	Pb(ppm)	Se(ppm)	8-Iso-$PGF_2\alpha$ (pg/ml)
Igbeta-Ewoama (n = 200)	0.226 ± 0.406	0.055 ± 0.122	0.331 ± 0.62	1.256 ± 2.34	1.060 ± 0.51	956.80 ± 365.36
Odi (n = 200)	0.017± 0.01	0.004 ± 0.011	0.081 ± 0.098	0.929 ± 0.314	0.037 ± 0.064	314.00 ± 202.35
P Value	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)

S = Significant

The effect of gender induced differences on the bioaccumulation of the measured metals and 8-Iso-$PGF_2\alpha$ in the two groups is presented in table 2. As shown in the table, males in Igbeta-Ewoama community had highest levels of Cadmium, Chromium and Mercury while females had the highest levels of Lead, Selenium and 8-Iso-$PGF_2\alpha$. These observed variations in concentrations among the two sub-groups were statistically significant in all the parameters $P<0.05$. Results from Odi community show that females had higher mean values of Cadmium, Chromium, Lead, Selenium and 8-Iso-$PGF_2\alpha$ while males had higher mean values of Mercury and Selenium than females. These observed differences were significant in all the measured metals except in 8-Iso-$PGF_2\alpha$, Cadmium and Lead at $P<0.05$. The mean concentrations of the measured heavy metals and 8-Iso-$PGF_2\alpha$ in the different age groups of Igbeta-Ewoama community are presented in table 3. Test of significance of these observed age induced differences using Tukey Post HOC analysis are presented in table 4. The highest mean level of Cadmium was observed in residents 31- 40 years old (0.425 ± 0.75ppm) while the least mean level (0.061± 0.03ppm) was observed in residents 51 – 60 years old. These values were not statistically different at $P<0.05$ (Table 4). The highest mean value of Chromium (0.131 ± 0.193ppm) was recorded in the age group 31- 40 years while the least value (0.003 ± 0.007ppm) was recorded among residents between 71-80 years.

Table 2: Heavy Metals and 8-Iso-$PGF_2\alpha$ Levels in Males and Females.

	Igbeta-Ewoama				Odi			
	Males n = 77	Females n =123	F value	P value	Males n = 106	Females n = 94	F value	P value
Cd(ppm)	0.271 ± 0.601	0.122 ± 0.18	6.629	0.011(S)	0.017 ±0.014	0.018 ± 0.006	0.412	0.522
Cr(ppm)	0.082 ± 0.161	0.004 ± 0.009	28.813	<0.0001(S)	0.004 ± 0.013	0.0041 ± 0.009	505.75	<0.0001(S)
Hg(ppm)	0.403 ± 0.92	0.166 ± 0.20	7.61	0.006(S)	0.109 ± 0.122	0.052 ± 0.06	16.89	<0.0001(S)
Pb(ppm)	0.755 ± 0.18	1.126 ± 0.29	101.43	<0.001(S)	0.732 ± 0.212	0.741 ± 0.18	0.103	0.748
Se(ppm)	1.039 ± 0.59	1.216 ± 0.42	6.123	0.014(S)	0.051 ± 0.07	0.022 ± 0.05	10.56	0.001(S)
8-Iso-$PGF_2\alpha$ (pg/ml)	904.29 ± 365.36	956.09 ± 410.56	0.008(S)	<0.0001(S)	259 ± 104	284.45±127	2.434	0.120

Table 3: Heavy Metals and 8-Iso-$PGF_2\alpha$ Levels in Subjects in Relation to Age in Igbeta-Ewoama Community.

Age Range	Cd(ppm)	Cr(ppm	Hg(ppm)	Pb(ppm)	Se(ppm)	8-Iso PGF_2□(pg/ml)
2– 10(n=30)	0.249 ±0.234	0.022 ±0.042	0.286 ± 0.403	0.689 ± 0.12	0.474 ± 0.109	848.60 ± 104.55
11 –20(n=34)	0.283 ± 0.26	0.080 ± 0.14	0.422 ± 0.41	1.079 ± 0.433	0.897 ± 0.46	1030 ± 118.6
21 – 30 (n=26)	0.388 ± 0.51	0.011 ± 0.034	0.542 ± 0.40	1.001 ± 0.193	0.900 ± 0.44	1088 ± 274.26
31 – 40 (n=30)	0.425 ± 0.75	0.131 ± 0.193	0.643 ± 0.856	0.717 ± 0.17	0.907 ± 0.68	1117 ± 284
41 – 50 (n=23)	0.125 ± 0.75	0.08 ± 0.11	0.341 ± 0.48	0.701 ± 0.15	1.00 ± 0.56	924.00 ± 209.06
51 – 60(n=25)	0.061± 0.03	0.05 ± 0.01	0.081 ± 0.068	0.719 ± 0.20	1.07 ± 0.34	730 ± 331.26
61 – 70(n=15)	0.094 ± 0.03	0.034 ± 0.01	0.092 ± 0.07	0.704 ± 0.13	1.14 ± 0.33	896.24 ± 309.61
71 – 80(n=17)	0.136 ± 0.23	0.003 ± 0.007	0.177 ± 0.09	0.876 ±	1.36 ± 0.308	949.80 ± 338.50

This difference was not statistically significant. Subjects 31-40 years old, had the highest mean concentrations of Mercury (0.643 ± 0.856ppm), while those 2 – 10 years old had the least values (0.286 ± 0.403). This age induced difference was not statistically significant. Residents between the ages of 11 – 20 years recorded the highest levels of Lead (1.079 ± 0.433ppm) while those 41 – 50 years had the least values (0.701 ± 0.15ppm). Selenium (1.36 ± 0.308ppm) was found to be highest in residents between 71 – 80 years old while the least value of 0.474 ± 0.109ppm was observed in those 2 – 10 years old. This observed difference was statistically significant

at P <0.05. Residents within the ages of 31 – 40 years had the highest mean concentration of 8-Iso-$PGF_2\alpha$ (1117 ± 284pg/ml) while least levels of 8-Iso-$PGF_2\alpha$ (730 ± 331.26) was observed in residents between the ages of 51 – 60 years. These observed differences were statistically significant at P<0.05.

The mean concentrations of the measured heavy metals and 8-Iso-$PGF_2\alpha$ in the different age groups of Odi community are presented in table 5. Test of significance of these observed age induced differences using Tukey HSD post HOC test are presented in table 6. The highest mean value of Cadmium (0.022 ± 0.01ppm), Mercury (0.097 ± 0.08ppm) and Selenium (0.098 ± 0.08ppm) were recorded in residents between the ages of 71 – 80 years old. Highest mean values of Chromium (0.038 ± 0.07ppm) and Lead (0.919 ± 0.25ppm) were recorded in residents 11 – 20 and 31 – 40 years respectively. Children 2 – 10 years old had the least mean values of Cadmium (0.012 ± 0.008ppm), Lead (0.502 ± 0.101ppm) and Selenium (0.045 ± 0.024ppm). Least mean values of Chromium (0.001 ± 0.001ppm) and Mercury (0.001 ± 0.001ppm) were observed in residents 71 – 80 and 21 – 30 years respectively. These observed differences in these age groups were statistically different at P<0.05. The highest mean concentration of 8-Iso-$PGF_2\alpha$ (408.04± 208.25pg/ml) was recorded in residents 71 -- 80 years old while residents that were between 2-10 years old recorded the least mean values (166.40 ± 34.). This measured difference was statistically significant at P<0.05. Table 7 shows the correlation between measured metals and 8-Iso-$PGF_2\alpha$ levels in Igbeta-Ewoama community. Significant correlation was observed between 8-Iso-$PGF_2\alpha$ and Mercury (P value <0.0001) as well as with Lead (P = 0.031). There was no significant correlation between 8-Iso-$PGF_2\alpha$ and the other measured heavy metals. Table 8 shows the mean values of measured liver function parameters in the study groups. It shows that residents in Igbeta-Ewoama community had the highest mean values of all the measured liver function parameters with the exception of albumin and total protein which were highest in the residents of Odi community. Results of the kidney function parameters measured in the study populations are presented in table 9. The table shows that highest levels of sodium (141 ± 2.2mmol/l), chloride (103 ± 3.6) and bicarbonate (29.8 ± 4.4) were recorded in residents in Odi. Residents in Igbrta-Ewoama had the highest levels of potassium (4.6 ± 0.5mmol/l), creatinine (104 ± 3.0µmol/l) and urea (6.1 ± 1.0mmol/l). These observed differences were statistically significant at p < 0.05.

Table 4: Comparison of Heavy Metals and 8-Iso-$PGF_2\alpha$ Levels in Subjects in Relation to Age in Igbeta-Ewoama Community.

Age Range	Cd (P Value)	Cr (P Value)	Hg (P Value)	Pb(P Value)	Se (P Value)	8-Iso PGF_2□ (P Value)
2-10 vs 11-20	1.0000	0.5595	0.9736	0.0000	0.0331	0.2112
2-10 vs 21-30	0.9702	1.0000	0.5781	0.0002	0.0349	0.0309(S)
2-10 vs 31-40	0.8812	0.0085	0.1323	0.9999	0.0210	0.0057(S)
2-10 vs 41-50	0.9902	0.6714	1.0000	1.0000	0.0083	0.9817
2-10 vs 51-60	0.8898	0.9892	0.8460	0.9999	0.0008	0.7930
2-10 vs 61-70	0.9627	0.0000	0.8907	1.0000	0.0002	0.9988
2-10 vs 71-80	0.9968	0.9996	0.9976	0.2606	0.0000	0.9437
11-20 vs 21-30	0.9876	0.1926	0.9751	0.8862	1.0000	0.9868
11-20 vs 31-40	0.9176	0.5186	0.5453	0.0000	1.0000	0.8590
11-20 vs 41-50	0.9319	1.0000	0.9988	<0.0000	0.9933	0.8226
11-20 vs 51-60	0.6547	0.9690	0.1440	0.0000	0.8635	0.0006(S)
11-20 vs 61-70	0.8314	0.0000	0.2005	0.0000	0.5556	0.5659
11-20 vs 71-80	0.9740	0.3070	0.7225	0.0980	0.0341	0.9742
21-30 vs 31-40	1.0000	0.0003	0.9892	0.0001	1.0000	0.9998
21-30 vs 41-50	0.5069	0.3256	0.8152	0.0002	0.9949	0.3303
21-30 vs 51-60	0.1849	0.8893	0.0117	0.0004	0.8819	<0.001(S)
21-30 vs 61-70	0.3393	0.0000	0.0202	0.0002	0.5886	0.1415
21-30 vs 71-80	0.6900	1.0000	0.2356	0.6785	0.0399	0.6862
31-40 vs 41-50	0.2856	0.6708	0.2935	1.0000	0.9959	0.1180
31-40 vs 51-60	0.0704	0.0879	0.0003	1.0000	0.8838	<0.001(S)
31-40 vs 61-70	0.1613	0.0000	0.0008	1.0000	0.5798	0.0362(S)
31-40 vs 71-80	0.4838	0.0037	0.0353	0.3312	0.0353	0.4069
41-50 vs 51-60	0.9998	0.9825	0.6020	1.0000	0.9996	0.1901
41-50 vs 61-70	1.0000	0.0000	0.6779	1.0000	0.9752	1.0000
41-50 vs 71-80	1.0000	0.4087	0.9711	0.3305	0.2986	1.0000
51-60 vs 61-70	1.0000	0.0000	1.0000	1.0000	0.9996	0.3606
51-60 vs 71-80	0.9997	0.8879	0.9987	0.4396	0.5501	0.1622
61-70 vs 71-80	1.0000	0.0000	0.9995	0.3406	0.8473	0.9985

Table 10 shows that there were statistically significant lower levels of Hb, WBC and Platelet measured in residents of the test community (Igbeta-Ewoama) ($P<0.05$) compared to the control community (Odi). Subjects in Odi community on the other hand, recorded the highest levels of Hb (12.02 ± 1.53g/dl), WBC (6.6 ± 0.63 $\times 10^9$/l) and Platelets (208.5 ± 7.52 $\times 10^9$/l). The length of bleeding time was prolonged in the residents of Igbeta-Ewoama community (13.16 ± 1.3 seconds) as compared to subjects from Odi community (12.74 ± 1.09). The difference in prothrombin time in the two populations was statistically significant.

Table 5: Heavy Metals and 8-Iso-PGF$_2\alpha$ Levels in Subjects in Relation to Age in Odi Community.

Age Range	Cd(ppm)	Cr(ppm	Hg(ppm)	Pb(ppm)	Se(ppm)	8-IsoPGF$_{2\square}$ (pg/ml)
2-10(n=27)	0.012 ± 0.008	0.021 ± 0.004	0.016 ± 0.014	0.502 ± 0.101	0.045 ±0.024	166.40 ± 34. 0
11- 20(n=36)	0.018 ± 0.004	0.038 ± 0.07	0.021 ± 0.02	0.612 ± 0.16	0.081 ± 0.011	184.00 ± 37.19
21-30(n=26)	0.016 ± 0.004	0.010 ± 0.002	0.001 ± 0.001	0.666 ± 0.17	0.056 ± 0.063	191.40 ± 50.30
31 - 40(n=32)	0.013 ± 0.003	0.012 ± 0.002	0.002 ± 0.002	0.919 ± 0.25	0.055 ± 0.05	209.60 ± 58.38
41- 50(n=20)	0.014 ± 0.002	0.009 ± 0.001	0.06 ± 0.04	0.900 ± 0.09	0.057 ± 0.004	234.40 ± 98.40
51- 60(n=21)	0.016 ± 0.02	0.006 ± 0.001	0.06 ± 0.04	0.90 ± 0.09	0.060 ± 0.04	264.10 ± 101.64
61- 70(n=20)	0.017 ± 0.01	0.004 ± 0.001	0.07 ± 0.08	0.864 ± 0.08	0.065 ± 0.07	306.50 ± 201.45
71- 80(n=18)	0.022 ± 0.01	0.001 ± 0.001	0.097 ± 0.08	0.818 ± 0.08	0.098 ± 0.08	408.04 ± 208.25

Table 6: Comparison of Heavy Metals and 8-Iso-PGF$_2\alpha$ Levels in Different Age Groups in Odi Community.

Age Range	Cd(P Value)	Cr (P Value)	Hg (P Value)	Pb (P Value)	Se(P Value)	8-Iso PGF$_{2\square}$ (P Value)
2-10 vs 11-20	0.1445	0.2479	0.9999	0.0567	0.1371	0.9980
2-10 vs 21-30	0.7280	0.8390	0.9458	0.0011(S)	0.9950	0.9891
2-10 vs 31-40	0.9999	0.9202	0.9509	<0.0001(S)	0.9962	0.7713
2-10 vs 41-50	0.9948	0.8275	0.0408(S)	<0.0001(S)	0.9945	0.3685
2-10 vs 51-60	0.7821	0.5861	0.0359(S)	<0.0001(S)	0.9776	0.0362
2-10 vs 61-70	0.5493	0.4385	0.0039(S)	<0.0001(S)	0.9052	0.0003
2-10 vs 71-80	0.0001(S)	0.0616	<0.0001(S)	<0.0001(S)	0.0011(S)	0.0000
11-20 vs 21-30	0.9881	0.0031	0.7311	0.8256	0.5970	1.0000
11-20 vs 31-40	0.2914	0.0039	0.7247	<0.0001(S)	0.4698	0.9744
11-20 vs 41-50	0.7428	0.0058	0.0706	<0.0001(S)	0.7344	0.6813
11-20 vs 51-60	0.9919	0.0011	0.0623	<0.0001(S)	0.8353	0.1118
11-20 vs 61-70	0.9999	0.0005	0.0068(S)	<0.0001(S)	0.9596	0.0012
11-20 vs 71-80	0.4583	.0001(S)	<0.0001(S)	<0.0001(S)	0.8294	0.0000
21-30 vs 31-40	0.9066	1.0000	1.0000	<0.0001(S)	1.0000	0.9980
21-30 vs 41-50	0.9950	1.0000	0.0011(S)	<0.0001(S)	1.0000	0.8709
21-30 vs 51-60	1.0000	0.9997	0.0009(S)	<0.0001(S)	1.0000	0.2758
21-30 vs 61-70	0.9999	0.9962	<0.0001(S)	0.0002(S)	0.9992	0.0077
21-30 vs 71-80	0.1082	0.8878	<0.0001(S)	0.0005(S)	.0278(S)	0.0000
31-40 vs 41-50	0.9999	0.9999	0.0007(S)	0.9998	1.0000	0.9916
31-40 vs 51-60	0.9311	0.9946	0.0006(S)	0.9998	1.0000	0.5967
31-40 vs 61-70	0.7636	0.9729	<0.0001(S)	0.8799	0.9978	0.0322
31-40 vs 71-80	0.0004(S)	0.6674	<0.0001(S)	0.0461(S)	0.0105(S	0.0000
41-50 vs 51-60	0.9963	1.0000	1.0000	1.0000	1.0000	0.9858
41-50 vs 61-70	0.9630	0.9992	0.9978	0.9933	0.9997	0.3830
41-50 vs 71-80	0.0191(S)	0.9605	0.0739	0.3865	0.0754	0.0000
51-60 vs 61-70	1.0000	1.0000	0.9976	0.9927	1.0000	0.9040
51-60 vs 71-80	0.1699	0.9973	0.0645	0.3637	0.1152	0.0009
61-70 vs 71-80	0.4110	0.9999	0.3996	0.9295	0.2757	0.0674

Table 7: Correlation between Measured Heavy Metals and 8-Iso-PGF$_2\alpha$ in Igbeta-Ewoama Community.

8-Iso-PGF$_2\alpha$	Cadmium		Chromium		Mercury		Pb(ppm)		Se(ppm)	
	R	P	R	P	R	P	R	P	R	P
	0.0074	0.298	0.060	0.399	0.528	<0.0001(S)	0.153	0.031(S)	-0.020	0.779

Table 8: Mean and SD Values of Liver Function Parameters in the Study Populations.

Study Community N = 200	ALT (U/I)	AST (U/I)	G-GT (U/I)	ALP (U/I)	ALBUMIN (G/L)	TP (G/L)	TB (µMOL/L)	CB (µMOL/L)
Igbeta-Ewoama	8.4 ± 1.1	11.8 ± 2	38.7 ± 3.6	26 ± 2	37.8 ± 2	63.3 ± 1	8.7 ± 0.06	2.4 ± 0.20
Odi	5.6 ± 1.3	9.5 ± 1.8	25.5 ± 4.2	22.3 ± 1	38.5 ± 2	63.8 ± 1.3	5.7 ± 0.1	2.0 ± 0.11
P Value	<0.0001(S)	<0.0001(S)	<0.001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.001(S)

S = Significant

Table 9: Mean and SD Values of Kidney Function Parameters in the Study Populations.

Study Community	Na^+ (mmol/l)	K^+ (mmol/l)	Cl^- (mmol/l)	HCO_3^- (mm0l/l)	Creatinine (µmol/l)	Urea (mmol/l)
Igbeta-Ewoama	139 ± 3.5	4.6 ± 0.5	101 ± 4.1	26.4 ± 3.8	104± 3.0	6.1 ± 1.0
Odi	141 ± 2.2	4.0 ± 0.5	103 ± 3.6	29.8 ± 4.4	97.7± 4.0	6.0 ± 0.7
P Value	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.0001(S)

S = Significant

Table 10: Mean and SD Values of Haematological Parameters in the Study Populations.

Study Community	Hb (g/dl)	Wbc ($\times 10^9$/l)	Platelet ($\times 10^9$/l)	Pt (seconds)
Igbeta-Ewoama	11.8 ± 1.4	5.4 ± 0.47	191.6 ± 5.8	13.16 ± 1.3
Odi	12.02 ± 1.5	6.6 ± 0.63	208.5 ± 7.5	12.74 ± 1.1
P Value	<0.0001(S)	<0.0001(S)	<0.0001(S)	<0.001(S)

S = Significant

DISCUSSION

Statistically significant high levels of bioaccumulation of heavy metals in the blood samples of residents in the area where oil producing activities are carried out were observed in this study as compared to the control community. The observed high heavy metal levels in the oil producing area may have resulted from spilled oil which contaminates both vegetation and aquatic life. Contaminated vegetation, aquatic animals and water are consumed by these residents causing an accumulation of metals in their bodies over time as the food chain progresses[31] as revealed by the results obtained. Males in the oil producing community (Igbeta-Ewoama) had the highest levels of all the metals and all the measured differences were significantly different at P<0.05. Some authors also reported significantly higher blood levels of lead and cadmium in males than in females[32], cadmium[33], lead[34], and nickel.[35] Conversely, some other authors[36] and[37] reported no difference between heavy metals blood concentrations in males and females. Evidently, gender is only one of many factors influencing blood heavy metal concentrations. An assessment of the effect of age differences on heavy metal bioaccumulation in the subjects shows that highest levels of cadmium, chromium and mercury were recorded in residents 31 – 40 years while highest levels of lead and selenium were recorded in residents between 11 – 20 years. The presence of high amounts of these metals in these younger ages may indicate serious medical concerns. This is as a result of the known toxic natures of these metals which could lead to eventual mortality of the residents with high concentrations of these metals before they get to older ages.

The serum level of the lipid peroxidation marker and indicator of oxidative damage (8-Isoprostaglandin $F_{2\alpha}$) was higher in residents of Igbeta-Ewoama than in the control community same as observed in the measured metals. This suggests that the metals influenced the concentrations of the oxidative stress biomarker 8-Isoprostanglandin $F_{2\alpha}$ as can be seen in the positive and significant correlations between them. The observed correlations indicate that Mercury and Lead are strong oxidative factors inducing the production of high levels of 8-Isoprostaglandin $F_{2\alpha}$ which is an indicator of oxidative stress. Lead is known to affect human immunity, blood cells, brain, kidneys, heart and male gonads.[38][39][40] Exposure to Cadmium can cause a variety of pathological alterations in several organs and tissues as well as induce diabetic complications, hypertension and osteoporosis.[41] Either redox-active or redox-inactive metals may cause an increase in production of reactive oxygen species such as the hydroxyl radical (HO-), superoxide radical (O_2^-) or hydrogen peroxide (H_2O_2). Increased generation of reactive oxygen species can overwhelm cells' intrinsic antioxidant defenses and give rise to oxidative stress. The results of this study suggest a higher probability of occurrence of renal impairment in residents of the oil producing area. This is supported by the significantly increased levels of serum urea and serum creatinine found in this community compared to those in the control community. This finding is in line with the findings by other researchers[42] who also reported significantly increased serum concentrations of urea, creatinine, potassium, uric acid and inorganic phosphate in subjects exposed to oil and gas pollutants. Crude oil has also been reported to cause destruction of the renal reserve capacity and also induced different severe pathological changes in the form of tubular necrosis in laboratory animals.[43]

The observed results of this work also suggest a possible higher incidence of liver impairment in residents of Igbeta-Ewoama communities due to significantly higher levels of AST, ALT, ALP, Gamma-GT, total bilirubin and conjugated bilirubin measured in the subjects of this community.

Measured hematological indices equally show that the residents in the oil producing area may be prone to anemia due to significantly lower levels of hemoglobin measured among them. They may also be prone to suppressed immunity as well as bleeding disorders as shown by the significantly lower levels of WBC, platelets and longer prothrombin time measured in their blood samples as compared to the control community. This is in line with the fact that crude oil exposures elicit adverse changes in hematological parameters. These changes affect blood and blood-forming cells negatively giving rise to anemia (aplastic), pancytopenia and leukemia.[44]

CONCLUSION

This study has revealed bioaccumulation of Cadmium, Chromium, Lead, Mercury and Selenium as well as increased levels of 8-Isoprostaglandin $F_{2\alpha}$ in the blood samples of subjects in Igbeta-Ewoama community. This supports the influence of heavy metals in the elicitation of reactive oxygen species and inducement of oxidative stress in humans.

These findings therefore suggest that prolonged exposure to Cadmium, Chromium, Lead, Mercury and Selenium may result in oxidative stress which is an indicator for likely risk for occurrence of diseases associated with oxidative damage such as atherosclerosis, respiratory disorders, neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, cancer, diabetes mellitus and inflammatory diseases among residents in Igbeta-Ewoama community.

RECOMMENDATIONS

With reference to the higher risks faced by people living in areas where oil producing activities are taking place and the poor regulation of oil producing activities in these places, it is strongly recommended that all safety standards and guidelines regarding safe oil exploration and production must be followed strictly in order to reduce and if possible avoid contamination of the environment with hazardous pollutants. A comprehensive investigation of the heavy metal content of all foods and water consumed in oil producing areas should be performed.

Since the production of isoprostanes is well documented to increase in direct proportion with the level of oxidative stress it will be valuable to employ its measurement in the assessment of exposure status of residents in environmentally impacted areas. The inclusion of antioxidants in the treatment of metal induced toxicity deserves further consideration since oxidative stress is scientifically proven to be an important mechanism for heavy metal toxicity.

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