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ORIGINAL RESEARCH

# Blood levels of some toxic metals in Human Immunodeficiency Virus (HIV) Type 1- infection

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**Abstract**

***Background*:** The introduction of antiretroviral therapy has reduced the mortality rate and increased the average life-expectancy of HIV-infected population. Infection probably due to the combination of the effects of environmental exposures and chronic inflammation and the role of toxic metals exposure and their health impact in infected individuals has been under-reported.
***Objective:*** To compare the plasma levels of cadmium (Cd), lead (Pb), mercury (Hg) and nickel (Ni) in HIV 1 -positive subjects receiving highly active anti-retroviral therapy treatment (HAART) and treatment-naïve subjects.
***Methods:*** The 300 study participants comprised 100 confirmed HIV-1 positive individuals on HAART, 100 HIV-1 positive HAART-naïve and 100 HIV-1 negative controls. Plasma levels of toxic metals were determined using inductively coupled plasma mass spectrometer (Agilent 7500, Norwalk, USA).
***Results:*** Plasma levels of toxic metals were significantly higher among HIV-infected subjects than controls ($p < 0.001$), with the only cadmium concentration being significantly higher ($p = 0.05$) among the HAART-treated subjects than HAART-naïve subjects.
***Conclusion:*** High toxic metal levels may lead to increased oxidative stress and adverse prognostic outcomes. Periodic evaluation of the blood levels of some toxic metals in HIV-1 infected individual is suggested and preventive strategies against environmental pollution should be adopted.

***Key words: Cadmium, Human Immunodeficiency Virus, Lead, Mercury, Nickel, Oxidative stress.***

## Introduction

Human Immunodeficiency Virus Type 1 (HIV-1) infection is a major public health challenge in Sub-Saharan Africa with associated significant morbidities and mortality. About 40 million individuals were reported to be infected with HIV world-wide with Sub-Saharan Africa having the highest burden. [1] The prevalence of HIV was estimated to be 3.2% among adults in Nigeria, thus making Nigeria the second country with the largest number of people living with HIV infection in Africa.[1]

The introduction of anti-retroviral therapy in the management of the infection had reduced the mortality rate among infected population thereby increasing their average lifespan. [2] The average life expectancy following HIV diagnosis in the United States reportedly doubled, increasing from 10.5 to 22.5 years [3] while the annual death rate declined from 1.69% in 1999 -2000 to 0.96% in 2007 - 2008. [4] The longer life expectancy in HIV-infection as a result of improved management of the disease has led to the upsurge in the occurrence of chronic non-infectious diseases such as cardiovascular diseases, diabetes mellitus, bone diseases, renal impairments, hypertension and malignancies. [5-7] Even though major advances have been made in understanding the biology of HIV infection and the

development of anti-retroviral therapy in the past decade, [8] the role of toxic metals exposure and their health impact on individuals living with HIV has been under-reported in Nigeria.

Exposure to environmental pollutants such cadmium (Cd), lead (Pb), mercury (Hg) and nickel (Ni) had been reported to increase the risk of many chronic diseases in the general population [9-11] and this may apply to HIV-infected populations. Toxic metals are widespread in the environment. Exposure to toxic metals is entirely unregulated in many developing countries, while little monitoring is conducted in developed countries. [12] The HIV-infected population generally has a lower socioeconomic status and lives in poorer communities, hence the higher risk of exposure to these toxins considering the correlation between poverty and environmental pollution. [12, 13] Moreover, environmental pollutants could adversely impact on the prognosis of HIV-1 infection probably due to the combined effects of environmental exposures and chronic inflammation. [13] Exposure routes may vary depending on the type of pollutant. Generally, exposure to hazardous toxic metals occurs via inhalation, ingestion, and dermal contact. Other sources of contact with pollutants include soil, dust, air, water (especially acid rain), and some food items. [14-15]
Fumes and soluble dusts of toxic metals are almost completely absorbed by inhalation. Adults absorb approximately 15% of an ingested dose through the gastrointestinal (GI) tract in contrast to 50% GI absorption in children. Gastrointestinal absorption is dependent on particle size and the solubility of the toxic compounds. [16] The cumulative health effect of exposure to these toxic metals may include several non-infectious chronic diseases. The blood levels of toxic metals in HIV-1 infected subjects are rarely monitored. It is not completely clear whether HIV-1 infected individuals are at a higher risk of exposure to environmental pollutants than the general population. [17] Studies that have evaluated the levels of toxic metals in HIV-1 infected subjects are sparse in Nigeria.

Therefore, this study sought to determine the blood levels of cadmium (Cd), lead (Pb), mercury (Hg) and nickel (Ni) in HIV 1-infected subjects and compare the levels in HAART-treated and HAART-naïve HIV-1 positive populations.

## Methods

*Selection of Study Participants*
The study participants were consecutively enrolled and they comprised 300 adult subjects - [100 confirmed HIV1-infected individuals receiving highly active antiretroviral therapy (HAART), 100 newly diagnosed HAART-naive HIV1-infected subjects and 100 HIV1 negative (apparently healthy) individuals]. The control group was recruited from among the staff and students of the University of Benin, Benin-City.

*Ethical Considerations*
The protocol for the study was approved by the Ethics Committee, Edo State Ministry of Health (Ethical code HM.1208/112 and dated 12th May 2016). The participants gave informed consent before recruitment into the study.

*Inclusion and Exclusion Criteria*
All the confirmed HIV-1-infected subjects attending the Antiretroviral Therapy (ART) Clinics at the Central Hospital, Benin-City who gave informed consent were included in the study. All HIV-1 sero-positive subjects who had an acute infectious illness (chest infections, bacterial endocarditis) and those who smoke cigarettes (which may affect plasma levels of toxic metals) were excluded from the study.

*Blood sample collection*
Four milliliters (4 ml) of blood sample were collected by venepuncture and the blood was dispensed into EDTA anticoagulant specimen bottle. The HIV sero-status was confirmed by testing the sample using two different test kits (Unigold® and Determine®). Thereafter, the plasma levels of the metals were determined using inductively Coupled Plasma Mass Spectrometer (ICP-MS)(Agilent 7500, Norwalk, USA) by adopting the methods of Fong *et al.* [18]

*Quality control*

Standards of the measured variables 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 being used all volumetric polyethylene (including the auto-sampler cups) and glass material were cleaned by soaking in 20% (v/v) HNO3 for 24 hours. 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 analysed. The measured cadmium level in whole blood was 3.05ng/ml with 3.38ng/ml as the certified value. The respective measured levels of lead and mercury in whole blood were 86.5ng/ml and 7.42ng/ml with 93.2ng/ml and 8.02ng/ml as the certified levels. In this study, nickel exposure was not controlled for and this may likely be a co-founder. The stability of calibration was checked periodically by analyzing the standard solution. Blank samples made from only reagents without sample were analysed to get rid of any background concentration metals in the system. Cyflow counter flow cytometer (Facs Flow Cytometer count system, Lincolnshire, IL, USA) was used to determine the $CD4^+$T-cell count.

*Statistical analysis*

Data generated from this study were analysed by the statistical software SPSS version IBM 21 (SPSS Inc., Chicago, IL, USA) for Windows. Continuous variables were expressed as mean with standard error of mean (SEM) and compared using the Student's t-test and analysis of variance (ANOVA). The correlation between continuous variables was done using the Pearson correlation coefficient. A *p*-value < 0.05 was considered statistically significant.

## Results

The study participants consisted of 100 confirmed HIV-1 positive individuals receiving HAART (40 males with mean age of 35.6 ± 0.6 years and 60 females with mean age of 32.8± 0.4 years), 100 newly diagnosed HAART-naive HIV-1 positive subjects (48 males with mean age of 33.2± 0.5 years and 52 females with mean age of 32.6 ± 0.2 years) and 100 HIV-1 negative, apparently healthy, subjects (controls, 50 males with mean age of 34.6± 0.2 years and 50 females with mean age of 32.0± 0.3 years).

Table I compares the mean measured blood levels of toxic metals in HIV-1 positive subjects with the control subjects and shows significantly higher ($p<0.001$) mean blood levels of toxic metals in HIV positive subjects compared with control subjects.

Table II compares the mean measured blood levels of toxic metals in HAART-treated HIV-1 positive subjects, HAART-naïve HIV-1 positive subjects and the controls. The correlations of blood levels of toxic metals with $CD4^+$ count as a marker of disease severity in HIV-infected subjects are shown in Table III. Blood levels of lead, cadmium and mercury decreased with increasing CD4 count while blood Ni level increased with increasing CD4 count but without statistical significance.

**Table I: Comparison of measured toxic metals in HIV positive subjects with controls (Mean ± SEM)**

| *Measured toxic metals* | *HIV-1 positive subjects(n=200)* | *HIV-1 negative subjects(n=100)* | *p-value* |
|---|---|---|---|
| **Age of subjects** | 33.5±0.7 | 33.3±0.3 | 0.5 |
| **Lead (µg/dl)** | 1.22±1.00 | 0.57±0.41 | <0.001 |
| **Cadmium (µg/dl)** | 0.62±0.27 | 0.10±0.01 | <0.001 |
| **Nickel (µg/dl)** | 0.89±1.19 | 0.11±0.01 | <0.001 |
| **Mercury (µg/dl)** | 0.08±0.00 | 0.04±0.00 | <0.001 |
| **$CD4^+$ (cells/µl)** | 479.6±43.2 | 789.5±81.2 | <0.001 |

**Table II: Comparison of measured toxic metals between HAART -treated and HAART -naïve HIV 1 -positive subjects and the controls (Mean ± SEM)**

| *Measured Toxic Metals* | *HAART-Naïve (n = 100)* | *HAART-Treated (n = 100)* | *HIV-Negative Controls (n = 100)* | *P values* |
|---|---|---|---|---|
| **Age of subjects** | 32.8 ± 0.5 | 33.9 ± 0.8 | 33.3 ± 0.3 | 0.2 |
| **Lead (µg/dl)** | 1.07 ± 0.85 | 1.38 ± 1.16 | 0.57 ± 0.41 | <0.001 |
| **Cadmium (µg/dl)** | 0.55 ± 0.26 | 0.68 ± 0.04 | 0.10 ± 0.01 | <0.001 |
| **Nickel (µg/dl)** | 0.95 ± 1.51 | 0.84 ± 0.11 | 0.11 ± 0.01 | <0.001 |
| **Mercury(µg/dl)** | 0.06 ± 0.02 | 0.09 ± 0.01 | 0.04 ± 0.00 | <0.001 |
| **CD4+ (cells/µl)** | 507.16 ± 41.45 | 452.30 ± 35.9 | 789.5 ± 81.2 | <0.001 |

**Table III: Correlation of $CD4^+$ cell count with measured toxic metals in HIV positive subjects**

| Correlation of Parameters | Correlation Coefficient (r) | *P* values |
|---|---|---|
| **Lead - $CD4^+$** | -0.142 | 0.16 |
| **Cadmium - $CD4^+$** | -0.169 | 0.09 |
| **Nickel - $CD4^+$** | 0.054 | 0.41 |
| **Mercury - $CD4^+$** | -0.095 | 0.35 |

## Discussion

The exposure levels of environmental pollutants in HIV-1 infected subjects are under-reported in Nigeria. The present study found significantly higher mean levels of measured toxic metals in HIV-1 infected subjects compared with the controls. The mean level of cadmium among HAART-treated subjects was significantly higher than the mean levels for HAART-naïve subjects, whereas the other metals did not show such significant difference between HAART-treated and HAART-naïve subjects. These findings are consistent with previous reports. [17, 19-20] It has been suggested that HIV-infected subjects may be more exposed to Cd compared to HIV negative individuals. [17] However, the reason for the higher Cd level in subjects on HAART treatment than HAART naïve is not clear, but may be attributed to the inability of the subjects to readily clear the toxic metals due to impaired renal and liver functions caused by HIV infection and the use of HAART. The subjects used in this study were neither smokers nor occupationally more exposed to pollutants than the controls. Socioeconomic factors may be cofounders in this instance, but there are insufficient data in the literature in support of this speculation. Chashchin *et al.* [19] reported that HIV-infected individuals may also be exposed to or accumulate some environmental pollutants such as lead and mercury in the body, [19] while Afridi *et al* observed that there were significantly higher mean levels of Cd, As, Ni and Pb in biological specimens of subjects with AIDS than controls. [20]

Some authors have previously attributed the higher blood levels of toxic metals in HIV-infected subjects to inability to readily clear these metals as a result of impaired renal and liver functions. [21] This finding may suggest that the assessment of toxic metals may be beneficial to HIV-1 infected subjects and intervention strategies to prevent exposure were suggested.[17] The clinical implications of higher blood levels of toxic metals in HIV-1 infected subjects are not completely clear, but it was suggested that they could be responsible for the increasing incidence of chronic non-infectious diseases in this population of individuals. [17] The relationship between toxic metal exposure and the risk of cardiovascular and respiratory diseases has been reported by several authors. [22-25] Other studies reported on the adverse effects of exposure to toxic metals on immune function. [26] HIV-1 infection is a disease characterized by generalized immune

activation [27] and elevated inflammatory activities. [28] It is suggested that high exposure to toxic metals may initiate or exacerbate the chronic diseases caused primarily by HIV-1 infection as well as the use of HAART.

One of the mechanisms by which toxic metals cause toxicity is by inducing oxidative stress, through the production of reactive oxygen species. On this basis, heavy metals are divided into redox-active and redox-inactive metals. Fenton-like reaction appears to play a major role in the oxidative stress observed in redox-active metal toxicity. [23, 28] The mechanism of toxicity of redox-inactive metals involves the depletion of cells' major sulfhydryl reserves. [22] Many proteins, both structural and others, have sulphur-containing amino acids which make them a potential target for these metals. In addition, several enzymes, including those in the antioxidant defense system, which protects cells from the deleterious effects of oxidative stress, unfortunately, contain sulfhydryl groups to which heavy metals can directly bind. These enzymes are inactivated if the sulfhydryl group is in their active site. [23] Furthermore, zinc, which usually serves as a cofactor of many enzymes, such as superoxide dismutase could be replaced by toxic metals, thereby making the enzymes inactive. [24] Therefore, metal-mediated oxidative damage occurs. Many metals could directly act as catalytic centers for redox reactions with molecular oxygen or other endogenous oxidants, producing oxidative modification of biomolecules such as proteins or DNA. This may be a key step in the carcinogenicity of certain toxic metals. [25, 29] Besides oxygen-based radicals, carbon- and sulphur-based radicals may also be produced. Nickel and chromium are two examples of metals which act, at least in part, by generation of reactive oxygen species or other reactive intermediates. [25] Alternatively, toxic metals could displace redox active essential elements from their normal cellular ligands (an ion, atom or molecules which donate a pair of electrons to a metal atom to form coordinate bond). This, in turn, may result in oxidative cellular damage. A good example is cadmium which is not redox active, but may cause oxidative stress through the release of endogenous iron, an element with high redox activity. [26] Metals in their ionic form can be very reactive and form DNA and protein adducts in biological systems. [30]

## Conclusion

The present study showed that measured toxic metals were higher in HIV-1 infected subjects, whether HAART-treated or HAART-naïve. The blood levels of the toxic metals in HAART-treated subjects appeared to be higher than the levels in HAART-naïve subjects. It is suggested that periodic assessments of toxic metal levels should be carried out among HIV-infected people and preventive strategies for environmental pollution may be helpful.

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