Blood Levels of Some Toxic Metals and Their Potential Health Impact in Human Immunodeficiency Type 1 Infected Subjects.

Abiodun EM, Mbonu I

Abstract

Background: The introduction of antiretroviral therapy in the management of immunodeficiency virus infection has reduced the mortality rate and increased the average life-expectancy of infected subjects. The prevalence of non-infectious chronic diseases and malignancies are also on the rise. Environmental pollutants could adversely impact on the prognostic outcomes of HIV-1 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 have been under-reported**.**

Objective: To evaluate the levels of cadmium (Cd),lead (Pb), mercury (Hg) and nickel (Ni) in HIV-1 infected subjects on highly active anti-retroviral therapy(HAART), HAART-naïve and discusses their potential health impacts.

Materials and methods: The study participants were 300 made up of 100 confirmed HIV-1 positive on HAART, 100 HIV-1 positive HAART-naïve and 100 HIV-1 negative controls. Measured toxic metal levels were determined using inductively coupled plasma mass spectrometer (Agilent 7500, Norwalk, U.S.A)

Results: Data indicated significantly higher ($p<0.001$) measured toxic metals in HIV positive subjects than controls, with levels in subjects on HAART higher than HAART-naive.

Conclusion: High toxic metal levels may lead to increased oxidative stress and adverse prognostic outcomes. Periodic evaluation of toxic metals in HIV-1 infected subjects is suggested and preventive strategies of environmental pollutants should be adopted.

Keywords: Human immunodeficiency virus infection, toxic metals, oxidative stress

Department of Medical Laboratory Science, School of Basic Medical Laboratory Sciences
College of Medical Sciences, University of Benin, Benin City.

Correspondence: Mbonu I, Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City.
Email:mathias.emokpae@yahoo.com

Introduction

Human Immunodeficiency type 1(HIV-1) is a major health challenge in sub-Sahara Africa, causing significant morbidity and mortality. The prevalence of the viral infection was estimated to be 3.2% among adults in Nigeria, thus making Nigeria the second country having the largest number of people living with the infection in Africa[1] The introduction of antiretroviral therapy in the management of the infection had reduced the mortality rate among infected subjected thereby increasing their average life-span.[2] It was reported that the average life expectancy after HIV diagnosis in the United States 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 among infected subjects as a result of improved management of the disease has led to the occurrence of non-infectious chronic diseases such as cardiovascular disease, diabetes mellitus, bone fractures, renal impairments, hypertension and malignancies.[5-7] Even though major advances have been made in understanding the biology of HIV infection and development of antiretroviral therapy in the past decade,[8] the role of toxic metals exposure and their health impact in individuals living with HIV has been under-reported.

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] which may also be true for HIV infected subjects. Toxic metals are widespread in the environment. Exposure to toxic metals is entirely unregulated in many developing countries and little monitoring is conducted in developed countries.[12] HIV-infected population generally have a lower socioeconomic status and live in poorer communities, which may consequently result in higher exposure to these toxins considering the correlation between area-level poverty and environmental pollution. Moreover, environmental pollutants could adversely impact on the prognostic outcomes of HIV-1 infection probably due to the combination of the effects of environmental exposures and chronic inflammation.[13] Exposure routes can vary depending on the pollutant. Generally, exposure to the hazardous toxic metals is most likely to arise through inhalation, ingestion, and dermal contact. In addition to direct occupational exposure, people can come into contact with these toxic materials and associated pollutants through contact with contaminated soil, dust, air, water (especially acid rain), and through food sources.[14-15] Fumes and soluble respirable dust 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 generally inversely proportional to particle size and directly proportional to the solubility of the toxic compounds.[16] The cumulative effect of these toxic metals could lead to several non-infectious chronic diseases and their levels in HIV-1 infected subjects are rarely assessed. 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 rare. This study therefore seeks to evaluate the levels of Cd, Pb, Hg and Ni in HIV-1 infected subjects on highly active anti-retroviral therapy (HAART), HAART-naïve and discusses their potential health impact.

Materials and Methods

Selection of Study Participants

The study participants were consecutively enrolled and comprised of 300 subjects that consisted of 100 confirmed HIV-1 positive individuals receiving highly active antiretroviral therapy (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) individuals recruited from among staff and students of University of Benin, Benin City (controls, 50 males with mean age of 34.6± 0.2 years and 50 females with mean age of 32.0± 0.3 years).

Ethical Consideration

The protocol of this study was reviewed and approved by the ethics Committee, Edo State

Ministry of Health (ethical code HM.1208/112 dated 12th May 2016). The participants gave informed consent before blood samples were collected.

Inclusion and Exclusion Criteria

All the confirmed HIV-1 subjects attending the antiretroviral therapy (ART) clinics at the Central Hospital, Benin City that gave consent were included in the study. All HIV-1 seronegative subjects who had an illness or infection (chest infections, bacterial endocarditis) or smoke cigarettes that may affect toxic metal levels as well as those who did not give consent were excluded from the study.

Sample Collection

The blood specimens were collected from the cubital fossa and were dispensed into EDTA anticoagulant specimen bottles.

The metal levels of the blood samples were determined by inductively Coupled Plasma Mass Spectrometer (ICP-MS)(Agilent 7500, Norwalk, USA) by adopting the methods of Fong et al.[18] Also the samples were confirmed for HIV infection.

Quality control

Standards of the measured variables were adequately prepared in order to check the reliability of the data. Standard sample for 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 h. They were finally rinsed with several washes of Milli-Q® water and dried in a polypropylene container. Certified reference materials from (Le Centre de toxicologie du, Quebec) were analyzed. 3.05 ng/mL was obtained as cadmium measured level from whole blood while 3.38 ng/mL is the certified value. 86.5ng/mL and 7.42ng/mL were obtained as lead and mercury measured levels from whole blood respectively while 93.2ng/mL and 8.02 were the certified value for lead and mercury respectively. In this study, we did not control for nickel exposure and this may likely be a co-founder to the results. The stability of calibration was checked periodically by analyzing the standard solution. Blank samples made from only reagents without sample were analyzed 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 $CD4^{+}$T-cell count.

Results

Table 1 shows the comparison of measured toxic metals in HIV-1 positive subjects compared with control subjects. Data indicate significantly higher ($p<0.001$) measured toxic metals in HIV positive subjects compared with control subjects.

Table 2 shows the comparison of measured toxic metals in HIV-1 positive subjects on HAART, HAART-naïve and controls.

Table 1: Comparison of measured toxic metals in HIV positive subjects with controls (Mean± SEM)

Measured toxic metals	HIV-1 positive subjects	HIV-1 negative subjects	p-value
Age of subjects	33.5±0.7	33.3±0.3	>0.05
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 2: Comparison of measured toxic metals between HIV-1positive subjects on HAART, HAART-naive and controls (Mean± SEM)

Measured toxic metals	HIV-1 Positive HAART-naïve N=100	HIV-1 positive on HAART N=100	HIV-1 negative controls N=100	P-value
Age of subjects	32.8±0.5[c]	33.9±0.8[c]	33.3±0.3	>0.05
Lead (µg/dL)	1.07±0.85[ac]	1.38±1.16[a]	0.57±0.41	<0.001
Cadmium (µg/dL)	0.55±0.26[ac]	0.68±0.04a	0.10±0.01	<0.001
Nickel (µg/dL)	0.95±1.51[ac]	0.84±0.11[a]	0.11±0.01	<0.001
Mercury(µg/dL)	0.06±0.02[ac]	0.09±0.01[a]	0.04±0.00	<0.001
CD4⁺ (cells/µL)	507.16±41.45[ab]	452.30±35.9[a]	789.5±81.2	<0.001

a=p<0.001; b=p<0.05; c=p>0.05

Discussion

The exposure levels of environmental pollutants in HIV-1 infected subjects are under-reported in Nigeria. The data presented in this study indicate a significantly higher (p<0.001) levels of measured toxic metals in HIV-1 infected subjects than HIV-1 negative controls. The level of cadmium in HIV-1 positive subjects on HAART was significantly higher (p<0.05) than HIV-1 positive HAART-naïve subjects while the increases in the other measured toxic metals were not statistically significant. The findings in this study are consistent with previous reports.[17,19-20] It was suggested that HIV infected subjects may be significantly more exposed to Cd compared to HIV negative individuals.[17] Chashchin et al.[19] reported that HIV-infected individuals may also be exposed to or accumulate some environmental pollutants such as Pb and Hg in the system,[19] while Afridi et al observed that there was a significantly higher mean levels of Cd, As, Ni and Pb in biological specimens of subjects with AIDS than

controls.[20] The observed higher levels of toxic metals could be due to inability of HIV infected subjects to readily clear these metals, because HIV infection and the use of HAART could impair renal and liver to detoxify and clear toxic metals from the body.[21-23] This finding may suggest that evaluation 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 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 group of individuals.[17] The relationship between toxic metal exposure and cardiovascular and respiratory diseases has been reported by several authors.[24,25-30] Others reported on the adverse effects of toxic metal exposure on immune function. [31-35] HIV-1 infection is a disease characterized by generalized immune activation[35-37] and elevated inflammatory activity.[38-40] It is suggested that high exposure to toxic metals may exacerbate or initiate chronic diseases caused primarily by HIV-1 infection as well as the use of HAART.

One of the well-known mechanisms toxic metals cause toxicity is by metal-induced 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. [41] The mechanism of toxicity of redox-inactive metals involves the depletion of cells' major sulfhydryl reserves. [42] Many proteins both structural and others have sulphur containing amino acid which makes them a potential target for these metals. Also, several enzymes including those in the antioxidant defense system which protects cells from the deleterious effects of oxidative stress are unfortunately containing sulfhydryl group to which heavy metals can directly bind. These enzymes are inactivated if the sulfhydryl group is in their active site. [43] 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. [44] 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.[45] Besides oxygen-based radicals, carbon- and sulphur-based radicals may also be produced. Nickel and chromium are two examples of metals that act, at least in part, by generation of reactive oxygen species or other reactive intermediates.[45] Alternatively, toxic metals could displace redox active essential elements from their normal cellular ligands (an ion, atom or molecules that donate a pair of electrons to a metal atom to form coordinate bond) which, in turn, may result in oxidative cellular damage. A good example is Cd, which is not redox active, but may well cause oxidative stress through the release of endogenous iron, an element with high redox activity.[46] Metals in their ionic form can be very reactive and form DNA and protein adducts in biological systems.[47]

In conclusion, our data indicate measured toxic metals were higher in HIV-1 infected subjects whether on HAART or HAART naïve. The levels of the toxic metals in those on HAART appear to be higher than HAART-naïve subjects. It is suggested that periodic assessments of toxic metal levels could be done and preventive strategies of environmental pollutants may be helpful.

References

1. Centers for Disease Control and Prevention. IV surveillance—United States, 1981-2008. Morbid Mortal Weekly Report. 2011;60: 689-693.
2. Losina E, Freedberg KA (2011) Life expectancy in HIV. BMJ 343: doi:10.1136/bmj.d6015.
3. Harrison KM, Song R, Zhang X. Life expectancy after HIV diagnosis based on national HIV surveillance data from 25 states,United States. J Acquir Immune Defic Syndr 2010;53: 124-130.
4. Smith C, Sabin CA,Lundgren JD, Thiebaut R. Data Collection on Adverse Events of Anti HIVdSG. Factors associated with specific causes of death amongst HIV-positive individuals in the D:A:D Study. AIDS, 2010; 24: 1537-1548.
5. Deeks SG, Phillips AN. HIV infection, antiretroviral treatment, ageing and non-AIDS related morbidity. BMJ 2009;338: a3172.
6. Sackoff JE, Hanna DB, Pfeiffer MR, Torian LV. Causes of death among persons with AIDS in the era of highly active antiretroviral therapy: New York City. Ann Intern Med 2006;145: 397-406.
7. Phillips AN, Neaton J, Lundgren JD. The role of HIV in serious diseases other than AIDS. AIDS 2008;22: 2409-2418.
8. Akinola FF, Akinjinmi AA, Oguntibeju OO. Effect of combined Anti-retroviral Therapy on selected trace elements and $CD4^+$ T-cells count in HIV positive persons in an African setting. AIDS Clinic Res 2012;3:10
9. Tellez-Plaza M, Guallar E, Howard BV, Umans JG, Francesconi KA, Goessler W et al. Cadmium exposure and incident of cardiovascular disease. Epidemiology 2013;24: 421-429.
10. Moon SS. Association of lead, mercury and cadmium with diabetes in the Korean population: the Korea National Health and Nutrition Examination Survey (KNHANES) 2009-2010. Diabet Med 2013;30(4):e143-138.
11. Rhee SY, Hwang YC, Woo JT, Sinn DH, Chin SO, Chon S et al. Blood lead is significantly associated with metabolic syndrome in Korean adults: an analysis based on the Korea National Health and Nutrition Examination Survey (KNHANES), 2008. Cardiovasc Diabetol 2013;12: 9. doi: 10.1186/1475-2840-12-9.
12. World Health Organization (WHO). Antiretroviral Therapy for HIV Infection in Adults and Adolescents. Recommendations for a Public Health Approach.2010 p. 108.
13. Hu H . Human Health and Heavy Metal Exposure. In: Life Support: Environ Human Health.2002;4:1–10.
14. Ivanov AV, Valnev-Elliston VT, Ivanova ON, Kochetkov SN, Starodubova ES, Bartosch B. Oxidative stress during HIV infection: mechanism and consequences. Oxidative Med Cell Longevit 2016;89:1-18.
15. Sackoff JE, Hanna DB, Pfeiffer MR, Torian LV. Causes of death among persons with AIDS in the era of highly active antiretroviral therapy: New York City. Ann Int Med 2006;145: 397-406.
16. Barbaro G, Klatt EC. Highly active antiretroviral therapy and cardiovascular complications in HIV-infected patients. Curr Pharm Des 2003;9: 1475-1481.
17. Xu X, Hu H, Dailey AB, Kearney G, Talbott EO,Cook RL. Potential Health Impacts of Heavy Metals on HIV-Infected Population in USA. PLoS ONE 2013; 8(9): e74288.
18. Fong BMW, Siu TS, Lee JSK,Tam S. Determination of Mercury in Whole Blood and Urine by Inductively Coupled Plasma Mass Spectrometry. J Analytic

Toxicol 2007; 31: 281-287.

19. Chashchin VP, Frolova NM, Sologub TV, Esadulenko EV. Influence of environmental and industrial immunotoxic hazards on clinical course of HIV-infection. Med Tr Prom Ekol 2010: 1-6.
20. Afridi HI, Kazi TG, Kazi N, Kandhro GA, Shah AQ, Baig JA et al. Evaluation of arsenic,cadmium, lead, nickel, and zinc in biological samples (scalp hair, blood, and urine) of tuberculosis and diarrhea male human imunodeficiency virus patients. Clin Lab 2011;57: 867-878.
21. Miro JM, Cofan F, Trullas JC, Manzardo C, Cervera C, Tuset M et al. Renal dysfunction in the setting of HIV/AIDS. Curr HIV/AIDS Rep 2012; 9:187-199.
22. Vallet-Pichard A, Mallet V, Pol S. Nonalcoholic fatty liver disease and HIV infection. Semin Liver Dis 2012; 32: 158-166.
23. Jones M, Núñez M. Liver toxicity of antiretroviral drugs. Semin Liver Dis 2012; 32: 167-176.
24. Alissa EM, Ferns GA. Heavy metal poisoning and cardiovascular disease. J Toxicol 2011;2011:870125. doi: 10.1155/2011/870125.
25. Kim DS, Lee EH, Yu SD, Cha JH, Ahn SC. Heavy metal as risk factor of cardiovascular disease--an analysis of blood lead and urinary mercury. J Prev Med Public Health 2005;38: 401-407.
26. Chaisemartin RA, Chaisemartin C. Compared effects of heavy metal ions (Cr+IV and Pb2+)on the respiratory intensity of 2 Crustacea Decapoda Brachyoura: Macropodia rostrata and Pachygrapsus marmoratus. CR Seances Soc Biol Fil 1976;170: 886-891.
27. Tellez-Plaza M, Navas-Acien A, Menke A, Crainiceanu CM, Pastor-Barriuso R. Cadmium exposure and all-cause and cardiovascular mortality in the U.S. general population. Environ Health Perspect 2012; 120: 1017-1022.
28. Lee MS, Park SK, Hu H, Lee S. Cadmium exposure and cardiovascular disease in the 2005 Korea National Health and Nutrition Examination Survey. Environ Res 2011;111: 171-176.
29. Messner B, Bernhard D. Cadmium and cardiovascular diseases: cell biology, pathophysiology, and epidemiological relevance. Biometals 2010;23: 811- 822.
30. Ozturk IM, Buyukakilli B, Balli E, Cimen B, Gunes S, Erdogan S et al. Determination of acute and chronic effects of cadmium on the cardiovascular system of rats. Toxicol Mech Methods 2009;19: 308-317.
31. Stepanova NV.Immunity status of children under the conditions of heavy metal-polluted city. Gig Sanit: 2003; 42-44.
32. Pölkki M, Kangassalo K, Rantala MJ. Transgenerational effects of heavy metal pollution on immune defense of the blow fly Protophormia terraenovae. PLOS ONE 2012; 7: e38832.
33. Vigneshkumar B, Pandian SK, Balamurugan K, Catalase activity and innate immune response of Caenorhabditis elegans against the heavy metal toxin lead. Environ Toxicol 2011; 28: 313–21.
34. van Ooik T, Pausio S, Rantala MJ. Direct effects of heavy metal pollution on the immune function of a geometrid moth, Epirrita autumnata. Chemosphere 2008;71: 1840-1844.
35. Sorvari J, Rantala LM, Rantala MJ, Hakkarainen H, Eeva T.Heavy metal pollution disturbs immune response in wild ant populations. Environ Pollut 2007;145: 324-328.
36. Bouman A, Schipper M, Heineman MJ,

Faas MM. Gender difference in the non-specific and specific immune response in humans. Am J Reprod Immununol. 2004; 52: 19–26.

37. Deitch EA, Ananthakrishnan P, Cohen DB, Xu DZ, Feketeova E, Hanser CJ. Neutrophil activation is modulated by sex hormones after trauma-hemorrhagic shock and burn injuries. Am. J. Physiol. Heart Circ. Physiol. 2006, 291, H1456–H1465.
38. Emokpae MA, Mrakpor BA. Do sex differences in Respiratory Burst Enzyme activities exist in Human Immuno-deficiency virus-1 infection? Med Sci 2016;4:19 doi.10.3390.
39. Angele MK, Schwacha MG, Ayala A, Chaudry IH. Effect of gender and sex hormones on immune responses following shock. Shock 2000, 14, 81–90.
40. Diodata MD, Knoferl MW, Schwacha MG, Bland KI, Chaudry IH. Gender differences in the inflammatory response and survival following haemorrhage and subsequent sepsis. Cytokine 2001; 14:162–169.
41. Liochev SI, Hausladen A, Fridovich I. Nitroreductase A is regulated as a member of the soxRS regulon of Escherichia coli. Proc Natl Acad Sci (Bichem) 1999;96:3537-3539.
42. Stohs SJ, Bagchi D. 1995. Oxidative mechanisms in the toxicity of metal ions. Free Radic. Biol Med 1995; 18: 321-336.
43. Quig D. Cysteine metabolism and metal toxicity. Altern Med Rev. 1998 ;3(4):262-270.
44. Marth E, Jelovcan S, Kleinhappl B, Gutschi A, Barth S. The effect of heavy metals on the immune system at low concentrations. Int J Occup Med Environ Health 2001;14: 375-386.
45. Bal W, Kasprzak KS. Induction of oxidative DNA damage by carcinogenic metals. Toxicol Lett 2002;127(1-3):55-62.
46. Valko M, Morris H, Cronin MTD. Metals, Toxicity and Oxidative Stress. Curr Med Chem 2005; 12:1161–1208.
47. Anetor JI, Iyanda AA, Akinseye I, Anetor GO. Strengthening Analytical Capability in Micronutrients: A Prophylactic Approach to DNA Repair Defects, Genome Instability and Carcinogenesis in Developing Countries. Ann Biologic Tech 2013;17: 560–561.

Citation: this article should be cited as. *Mathias Abiodun Emokpae, Ikenna Mbonu*. Blood Levels of Some Toxic Metals and Their Potential Health Impact in Human Immunodeficiency Type 1 Infected Subjects. Afr. J. Trop. Med. & Biomed. Res 2019; 4 (2): 26-33