ejpmr, 2017,4(12), 91-95

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

SOME HEAVY METAL ASSAY IN URINE OF BREAST CANCER AND BREAST CANCER FREE PATIENTS IN PORT HARCOURT, NIGERIA

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Article Received on 13/10/2017 **Article Revised on 02/11/2017** **Article Accepted on 23/11/2017**

ABSTRACT

Urine levels of Copper (Cu), Cadmium (Cd), Chromium (Cr), Lead (Pb), Nickel (Ni), and Zinc (Zn) were assayed in 150 patients using Atomic Absorption Spectroscopy (AAS). 100 of these test patients were pathologically and histologically positive for breast cancer and while 50 (not positive for breast cancer) served as control. The study was carried out to ascertain if possible, a link between exposure to these metals and the recent upsurge in the incidence and burden of likely environmentally induced breast cancer in Port Harcourt, Nigeria. The result showed elevated and significant ($p<0.05$) levels of all the metals analysed when company to the controls.

KEYWORDS: Urine, heavy metals, AAS, breast cancer, environment.

INTRODUCTION

Over the years, enhanced knowledge of the significance of heavy metals as regards human health has led to approach in technology that eased their analysis in body fluids and tissues (Sandstead, 1985) and the increased attention in the knowledge of the major role heavy metals are involved in different disease states has led to several studies in understanding the probable link. However, heavy metals are a small part of the biology of the human systems responsible for essential biochemical processes in very reduced concentrations. Also, several diseases whose cause was not known before now have been linked to variation in the levels of these metals, either in excess or deficiency. Due to the basic importance of heavy metals in several functions of the human body, it is logical to hypothesize that their variation in the body may enhance some biochemical processes such as creation of reactive oxygen specie (ROS), which could lead to diseases such as cancer, stroke, diabetes etc. (Halliwell *et al.*, 1993).

Breast cancer has overtaking cervical cancer as the most diagnosed cancer in women in most part of the world (Parkin *et al.*, 2001), Port Harcourt, Nigeria included. Even when the incidence is lower in developing countries, the burden and mortality rate of the disease is far greater as a result of poverty, education, poor health facilities leading to poor and late diagnosis and cultural beliefs. The various known risk factors for breast cancer; female sex, age, hormones, early puberty (menarche), late menopause, null parity, having the first child at a late age, BRCA genes, family history of breast or any cancer (Harris *et al.*, 1992) have been established to not really clarify the high incidence and differences in geographical location linked to the disease (Strugeon *et al.*, 1995), as research has shown that half the women with breast cancer do not have the already known and proven risk factors besides the female sex and age (Madigan, 1995). Breast cancer as a disease has been known to be hormone (Oestrogen and Progesterone) dependent but heavy metals as environmental factors act as endocrine disrupters at cellular or subcellular levels via conflicting mechanisms, one of such could be the relationship between heavy metals and hormones that control the metabolic process of the human body (Megalova *et al.*, 1999).

Minerals, metals and their compounds included occur in nature and are an intricate part of the environment. Many are important for normal physiological function while others have shown to be toxic to normal body process at an elevated level (John & Darbre, 2006). Several metal compounds in the environment have shown that they are able to bind to cellular oestrogen receptors and imitate the actions of those physiological oestrogens, often referred to as xenoestrogens or metalloestrogens. These metalloestrogen (metal oestrogen) includes Chromium, Zinc, Lead, Mercury, Nickel, Cadmium, Barium, Cobalt, Copper, Aluminum, Arsenite, Selenite, and Tin etc. The potential for these metal ions to increase the burden of oestrogen signalling in the human breast is broad (John & Darbre., 2006). With the advent of industrial revolution individuals have found increasing number of uses for various metals in such areas as industry, agriculture, medicine, etc. These activities have no doubt enhanced exposure to metal related occupational. These

metalloestrogens considered harmful pollutants especially in a higher than normal and sustained concentration in the soil and water are not degraded easily making elimination difficult (Pan *et al.,* 2010). These compounds enter the body through air, food, water and cosmetics or through dermal exposure, through the plasma membrane to the cell in order to cause toxicity (Wood *et al.,* 2011).

In the developing world, Nigeria included, lakes, rivers and streams have been heavily contaminated with pollutants from the environment, heavy metals included due to the steady discharge of untreated and unregulated industrial waste, agricultural waste water and unhygienic human activities (Cleaver *et al.,* 2007). Interestingly, these activities have been implicated to increased levels of these toxic metal compared to what is considered safe for the generation and the consequence is myriad of ill health and diseases, cancer included. Sustained exposure to various cancer causing agents, metals include elicits the formation of reactive oxygen species, ROS. ROS is known to be vital in the origin of several human cancers. They are implicated in mutation and damage to DNA (Collins, 1999).

MATERIALS AND METHODS

Study Population

150 patients, 100 patients, serologically and histologically confirmed as positive for breast cancer of either ductal carcinoma, ductal carcinoma in situ and/or lobular carcinoma. These females attended the Surgery clinics of University of Port Harcourt Teaching Hospital, Braithwaite Memorial specialist Hospital and Meridrien Hospital, Port Harcourt with a palpable lump in the breast. 50 patients, who served as control, were individuals with no prior proven individual or family history of either breast or any other type of cancer excluded by thorough physical examination and serum uric acid evaluation.

Informed consent was obtained from each patient and ethical approval was also obtained from the ethics committee of the various institutions before the commencement of the study. The patients were all recruited and implicated at the pre-surgical or any treatment stage at the treatment centres.

Examination of the patients

A detailed history of all the subjects was recorded. This includes age of menarche, parity, duration of breastfeeding, family history if any, of breast cancer or any other type of cancer, place and duration of residence, occupation, use of tobacco as well as alcohol among other things.

Detailed physical and clinical examination was performed on both breasts. The physical characteristics of lump on the breast and surrounding area, nipple examination, lymph node involvement and any sites of bone pain were properly examined. A serologically and histologically clinical diagnosis of breast carcinoma alongside the clinical stage was established.

Collection of Samples

Samples were collected from the patients at the treatment centres. Urine samples were collected in sterile urine sample bottles.

Experimental Design

There were two study groups, A and B. Group A consisted of confirmed breast cancer patients while group B consisted of non cancerous patients with no risk, non proliferation benign breast diseases. Group B served as control.

Sample treatment

Urine samples were collected and preserved in the laboratory refrigerator before analysis.

Biomedical Parameters

The parameters assayed were;

a) Levels of metals (Cd, Pb, Zn, Cr, Ni and Cu) in urine samples.

Analysis of heavy metals

Urine copper, zinc, chromium, lead, nickel and cadmium were determined using a solar thermo-elemental flame atomic absorption spectrometer (FAAS) (Model S4-71096) American standard. The urine samples were first treated by digestion with nitric and perchloric acids. Samples for elemental assays of different matrices, either organic or inorganic needs partial or absolute dissolution of the samples before assays with the instrument. The digested samples were assayed for heavy metals with FAAS with appropriate wavelengths and hollow cathode lamps.

Statistical analysis

Graphpad prism version 7 and Stata statistical data analysis, version 12 were used. 2-group Hoteling's T-squared test was used to test for significance (@<0.05).

RESULTS

Table 1 – Mean + SD of urine heavy metals for test and control.

Parameters Subjects	Cu(µg/l)	Zn(µg/l)	Cd(µg/l)	Ni(µg/l)	Pb(µg/l)	Se(µg/l)	Cr(µg/l)
Test	0.537 ± 0.99	0.801 ± 0.48	0.295 ± 0.34	1.397 ± 0.92	2.175 ± 0.70	0.019 ± 0.03	1.024 ± 0.94
Control	0.057 ± 0.06	0.084 ± 0.07	0.118 ± 0.32	0.186 ± 0.26	0.559 ± 0.56	0.446 ± 0.39	0.331 ± 0.34
P value	0.0008	<0.0001	0.0030	<0.0001	<0.0001	<0.0001	<0.0001
T value	3.427	10.47	3.015	9.132	14.27	10.88	5.021
Remarks	S	S	S	S	S	S	S

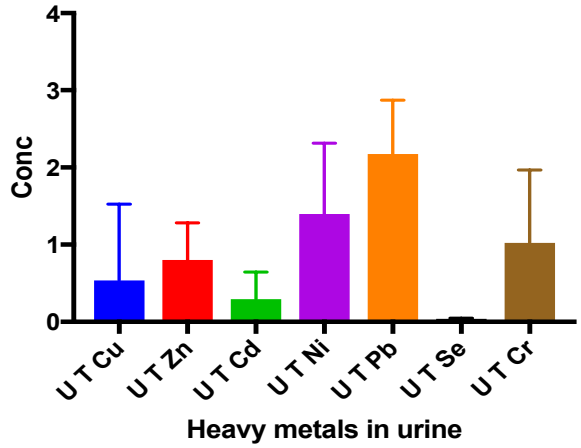

Fig 1.

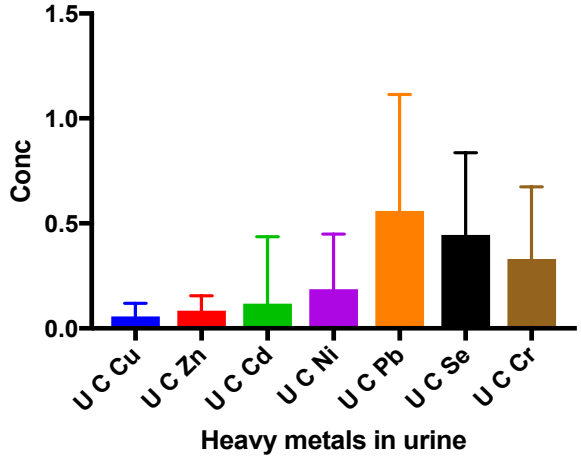

Fig 2.

DISCUSSION

Cancer, a group of disease known to be initiated by several factors that assumes unregulated growth of cells followed by cell invasion, spread and secondarily, neoplasm formation at the origin or sites far from the origin aid through angiogenesis. These processes are always part of an immune response and for a tumour to grow larger than normal, angiogenesis is required (Bryne *et al.*, 2013). Table 1 shows that the metals investigated showed statistical significance when compare to the controls in all category. This shows that besides the major risk factors for breast cancer, heavy metals, through oxidative processes could lead to cancer. Cu is vital for the function of many enzymes (Denoyer *et al.*, 2015), hence it is needed for attributes required in cancer growth such as proliferative immortality, angiogenesis and metastasis and also an initial cofactor in the expression, operation and release of key activators of angiogenesis through multiple mechanism (Denoyer *et al.*, 2015). Copper could elicit a pro-angiogenetic response (Gupte and Mumper, 2009) by binding direct to angiogenetic factors that improve the appearance of related factors (Kenneth *et al.*, 2014). The possibility of Cu to move between its two states of oxidation, Cu^{+} and Cu^{2+} could result to harmful effects on cell function in initiating high ROS, which is able to make hydroxyl and other radicals that adversely reshapes proteins, lipids and nucleic acids (Brem *et al.*, 1990).

Significant ($p<0.000$) levels of cadmium are also seen in the urine of the test patients when compared to the control. In the study by Ionescu *et al.*, (2006), they found that the mean urinary level in breast cancer patients was raised significantly than patients with benign breast disease. McElory *et al.*, (2006), reported a raised significant level by two-fold of breast cancer risk in women with raised urinary cadmium levels. Strumylaite *et al.*, (2011) found raised significant urinary cadmium level in 57 breast cancer patients as against 51 benign breast disease patients. This present study agrees with the ones outlined above based on the result obtained. Cadmium, with a half-life of over 10 years (Nawrot *et al.*, 2006), is unique among other heavy metal pollutants because urinary Cd is a long-term biological indicator of sustained exposure and establishing absolute body burden of the metal (Satarug *et al.*, 2010). Nickel levels were also significantly ($p<0.000$) raised in the test patients when compared to non-test patients. Nickel is necessary for the catalytic activity of some plant and bacterial enzyme. Elemental nickel could be vital for life at very low concentration, which is usually non-toxic (ATSDR, 2005). This study agrees with the role of chromium in breast cancer carcinogenicity. Ionsescu *et al.*, (2008), found highly vital nickel collection in 20-breast cancer tissue biopsies compared to controls. Hence, Nickel has been known to inhibit the repair of damaged DNA, it is then suggested that collection of nickel in breast tissue may be closely associated to malignant growth action. The binding of nickel to the oestrogen receptor appears to elicit the demonstration of genes linked with cell development (Martin *et al.*, 2003). Though few studies have analyzed the outcome of nickel on gene expression in mammary cells, the outcome of nickel has been widely studied in other cell types (Kawata *et al.*, 2009). Similarly, Kilic *et al.*, (2004), analysed hair samples from breast cancer patients and cancer free group. They compared the average chromium content of breast cancer with those of control group. Their results showed an elevated average concentration of chromium for breast cancer patients compared with the control group, making hair sample analysis one of many tools in the determination of metal status in the human body. Wang *et al.*, (2000), reported that the two different chromium reactive states; Cr (V) and Cr (IV) are involved in the creation of OH radicals that lead to DNA breaks, base modification, lipid peroxidation and nuclear transcription factor NF-kB initiation and deactivation of p53.

Zinc levels were also significant (<0.05) in the study. Lee *et al*, (2003), indicated that changed zinc homeostasis is attributed for increased zinc levels in breast tissues and body fluids of breast cancer patients. Its homeostasis controlled by protein molecules termed zinc transporters at the time of influx, efflux and

retention to ensure adequate levels are maintained. In their study, N-methyl-N-nitrosourea (MNU)-induced rat mammary tumour showed zinc build up in the mammary tumours due to a low Zn7-1 (Zinc efflux transporter) appearance and high metallothionen (zinc storage protein) appearance. The increased level of zinc could lead to growth of breast cancer. This is because scientist have also indicated that zinc is involved in disease states such neuro-degeneration, inflammation, diabetes etc. they found a reduction in CK2, a protein that opens one transporter, ZIP7 to let zinc expel and flow. High levels of intracellular zinc and ZIP7 transporter were discovered in tamoxin-resistant breast cancers (Taylor *et al.*, 2012).

From the table 1, lead (Pb) also showed significant ($p<0.05$) higher values in the breast cancer patients than those of the controls. The potential of lead to act as a potent oestrogen indicate that it could be a vital class of endocrine disruptors (Martin *et al.*, 2003). Lead in the environment is subjection is toxicologically important in the generation of ROS, which cause oxidative damage, i.e. the direct participation of lead in free radical reactions leading to elevated risk of breast cancers or tumours. This pathway may potentiate and account for the suspected environmentally induced cancer prevalent in these subjects.

CONCLUSION

The high levels of copper, zinc, cadmium, nickel, lead and chromium in the urine of the cancer subjects studied is a pointer that these heavy metals could be implicated in the high prevalent rate of breast cancer in the subjects studied.

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