Element Levels in Plasma of Cancer Patients

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Abstract
This study investigated the levels and the trend of some plasma elements in cancer patients. A total number of seven hundred (700) patients with different types of cancer (leukemia, ovarian, prostate, breast, colon, cervical, uterine, ovarian) attending University Teaching Hospitals and Federal Medical Centers in Southwestern Nigeria, and three hundred (300) healthy subjects who served as control subjects were examined. The plasma levels of Zn, Fe, Ca, Mg, Mn and Cu were determined by atomic absorption spectrophotometry while Na and K, were analysed using flame emission photometry. The alterations observed in plasma profile of elements of the cancer patients when compared with the control may be a useful indicator in the detection of the initial changes occurring in neoplastic cells. A significant increase (p<0.05) was recorded in the plasma level of lead and cadmium when compared with the control and among the cancer types which suggests their involvement in the generation of reactive oxygen species which could lead to lipid peroxidation, DNA damage and alteration in gene expression.

Keywords: cancer, elements, plasma, alteration, patients.

Introduction
Cancer known medically as a malignant neoplasm is a term for a large group of different diseases, all involving unregulated cell growth. Cancer is not just one disease but many diseases that all have to do with cells. It results from abnormal growth and spreading of cells (Anand et. al., 2008). Normal body cells grow, divide, stop growing and over time, they die. Unlike normal cells, cancer cells just continue to grow and divide out of control and do not die when they are supposed to (Murgia et. al., 2006). Sometimes, cancer cell breaks away from the original tumor and travels to other areas of the body, where they keep growing and can go on to form tumors. The spread of a tumor to a new place in the body is called metastasis. Not all tumors are cancerous, tumors can be benign or malignant (Kushi et. al., 2006). Benign tumors are not cancerous. They can often be removed, and in most cases, they do not come back. Cells in benign tumors do not spread to other parts of the body (Merlo et. al., 2006).

The immune system, which are the immunoglobulin’s, cell mediated immunity, phagocytosis complement, lysosome interferon, metabolic function, hormones, metabolic and respiratory alkalosis are the natural mechanism. Zinc is essential for normal development and maintenance of the thymus. Reduced antibody synthesis, during zinc deficiency relates in part to interferences with T-lymphocyte helper function. Zinc promotes B-cell function by acting as a mitogen for animal and human lymphocytes (Cunzhi et. al., 2003). Zinc is essential for the normal bactericidal and phagocytic function of granulocytes. Zinc essential role in protein synthesis make it essential for normal complement production Acrodermatitis enteropathical, failure to absorb zinc because of an inborn error of metabolism, is marked by all the above defects. Blood and serum from patient generally show subnormal zinc levels. Zinc deficiency depresses killer cell activity. Zinc deficiency promotes cancer by inhibiting normal vitamins A, lipid metabolism and DNA repair (Navarro-Alacon and Lopez-Martinez 2000). Molybdenum has anti-euplastic properties molybdenum and also manganese prevent the formation of some experimentally induced cancer manganese may augment bactericidal action of antibiotics. Elevated serum copper levels have been described in patients with a variety of carcinomas. Copper had been reported to be a biological antagonist of vitamin C and zinc (Desch et. al., 2005).

Potassium, the most abundant intracellular cation, is essential for the life of the organism. Potassium is obtained through the diet (Durlach et.al.,2000). Potassium homeostasis is maintained predominantly through the regulation of renal excretion. The most important site of regulation is the distal nephron, including the distal convoluted tubule, the connecting tubule, and the cortical collecting tubule, where aldosterone receptors are present (Bios, 2003). Potassium is predominantly an intracellular cation; thus, serum potassium levels can be a very poor indicator of total body stores. Potassium moves easily across cell membranes; therefore, serum potassium levels reflect the movement of potassium between intracellular and extracellular fluid compartments as well as total-body potassium homeostasis (Fuster et. al., 1992).

Sodium reabsorption through epithelial sodium channels (ENaC) located on the apical membrane of cortical collecting tubule cell, is driven by aldosterone and generates a tubular lumen negative electrical potential, driving the secretion of potassium at this site through specific potassium channels called the renal outer medullary K channels (ROMK) (Doyle et. al., 2007).

Sodium (Na⁺), a major cation is considered together with water because their homeostatic mechanisms are closely related and inter-dependent. When osmotic pressure increases uncontrollably to a point of saturation,
an increase of ECF sodium under this condition may cause the obstruction of the uterus and the uterine cavity, distorts menstruation cycle and consequently results to the cancer of the uterus which is better described as Endometrial cancer (Harriss et al 2009). Sodium balance in the body is greatly dependent on the rate of renal sodium excretion. The renal excretion of sodium is controlled in the final instance by the glomerular filtration Rate (GFR), aldosterone, and the atrial natriuretic peptide (ANP). GFR influences the tubular absorption rate of sodium filtered by the glomerulus (~2500mmol/day). Aldosterone increases the distal tubular reabsorption of Na+ and the excretion of H+ and K+.

Zinc had been reported to inhibit the development of cancer and low serum zinc had been associated with several forms of cancer. When the tumor is associated with tumors rich in zinc, return to normal level was associated with a favorable prognosis (Anand et al., 2008). Aqueous zinc acetate injected intraperitoneally prevented tumor growth in 50 to 70 percent of male mice previously inoculated with L1210 leukemia cells. Subcutaneous injections in a different strain did not prevent tumor growth but significantly increased mean survival (Kuo et al., 2002). Zinc deficiency has been shown to increase the susceptibility in animals to chemical carcinogens that produce esophageal cancer (Manhart and Koutsy 2002). Low zinc levels due to phytate consumption have promoting effects on precancerous esophageal tumors. Populations having a usually high risk for esophageal cancer tend to subsist on zinc deficient diets, predominantly cereal and have a low intake of animal products found that minimal levels of zinc intake which ensure optimal body growth rate are inadequate to provide maximal resistance to esophageal carcinogenesis. Zinc may prevent cancer via the metabolism of high density lipoprotein (HDL), vitamin A and DNA synthesis (Jemal et al., 2011). High levels of zinc supplementation in normal subjects, 440mg of zinc sulfate per day for five weeks, result in a 25 percent decrease below baseline levels of HDL (Al- Ebraheem et al., 2010).

Manganese had been found comparable to molybdenum in concentration in animal tissues and fluids of all species so far examined. Sarcoma incidence of 96 to 100 percent of rats receiving N1352 was reduced to 63 percent by manganese dust. Manganese alters the subcellular distribution of carcinogenic nickel (Desch et al., 2005). This particular anti-carcinogenic effect was Local, specifically related to the site of injection rather than systemic. Manganese may exert its anti-carcinogenic effects by activating superoxide dismutase would be inactive and accumulation of superoxide anion could be mutagenic (Kuo et al., 2002).

Iron can be carcinogenic in three ways first, ferric ions are reduced by superoxide and the ferrous product is re-oxidized by peroxide to regenerate ferric ions and to yield hydroxyl radicals (Al-Ebraheem et al., 2010). Reactive oxygen species such as hydroxyl radicals, when generated in close proximity to DNA can alter normal cells by causing point mutation, DNA cross linking and DNA strand breaks. Oxygen free radicals also can cause conformational changes in nuclear and cytoplasmic membranes by inducing lipid predication. Secondly, in addition to the initiation of the cancer process, iron can bolster the growth of neoplastic cells by suppressing host defenses. Furthermore the tumoricidal action of macrophages is suppressed by phagocytized erythrocytes, erythrocyte lysate, hemoglobin, ion dextran or iron salts, but not by erythrocyte ghost membrane, latex spheres myoglobin (Kuo et al., 2002). The third mechanism whereby iron can be carcinogenic is that of functioning as an essential nutrient for unrestricted tumor cells multiplication. Although normal and cells have a similar qualitative requirement for iron, the unrestricted proliferation of tumor cells would be expected to require an enhanced and probably diversified, supply of the metal. In 1972, Holley proposed that the crucial change in a malignant cell is an alteration in the cell surface membrane that results in internal concentration of nutrients that to provide maximal resistance to esophageal carcinogenesis. Zinc may prevent cancer via the metabolism of high density lipoprotein (HDL), vitamin A and DNA synthesis (Jemal et al., 2011). High levels of zinc supplementation in normal subjects, 440mg of zinc sulfate per day for five weeks, result in a 25 percent decrease below baseline levels of HDL (Al- Ebraheem et al., 2010).

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Copper is an important trace metal which plays a fundamental role in the biochemistry of the human system. It is an important component of several metallo-enzymes including tyrosine and dopamine hydroxylase (Liversay, 2003). Copper has been suggested to play an important role in several disorders (Wallwork, 1987). Copper ions and copper complexes react with hydrogen peroxide to form hydroxyl radicals that cause an irreparable damage to protein RNA and DNA (Arinola and Charles-Davies, 2008). Critical proteins such as cytochrome oxidase, zinc–copper, superoxide dismutase, lysyl oxidase and several transcription factors require copper for activity. Consequently, complex systems for acquiring and regulating copper influx and efflux have evolved. In the yeast Saccharomyces cerevisiae, for example, several essential enzymes are copper dependent. S. cerevisiae has therefore developed an elaborate system to ensure adequate levels of copper. In an environment where copper is scarce, genes are activated to enhance copper uptake. In mammals, copper balance is established by maintaining equilibrium between copper absorption from dietary intake and copper excretion in the stool (Bikfalvi and Bicknell, 2002). Based on the tightly controlled homeostasis of copper in primitive organisms, the hypothesis that copper availability was a primitive growth regulator, and that this regulatory aspect of copper has been maintained throughout evolution had been developed (Brewer 2001a). In the human and other mammals, the first observable evidence of copper deficiency is a drop in the level of blood ceruloplasmin (Cp) (Brewer et al., 2000). Cp is a copper-containing protein secreted by the liver into the blood.
The copper in Cp accounts for about 90% of the total plasma copper. As copper availability to the liver decreases, the liver decreases production and secretion of Cp. Thus, plasma Cp is a good surrogate marker of body copper status. There are no known harmful effects of a decreased level of Cp, or the decreased availability of copper, associated with moderately reduced Cp levels (defined as a reduction of up to 80%). As the Cp becomes very low, however, clinical evidence of copper deficiency emerges. The first sign is bone marrow suppression manifested as anemia, with or without leukopenia (Brewer et. al., 2000). The next stages of copper deficiency may involve connective tissue defects and possibly decreasing bone density if copper deficiency persists. These intermediate stages of copper deficiency are not well described in the human. A late stage of copper deficiency, which has been seen only very rarely in patients, involves a severe neuropathy in addition to pancytopenia (Bikfalvi and Bicknell, 2002).

Selenium is a vital antioxidant, especially when combined with Vitamin E. As an antioxidant, selenium protects the immune system by preventing the formation of free radicals (unbalanced molecules), which can damage the body. Selenium and Vitamin E act together to aid in the production of antibodies and to help maintain a healthy heart. This trace element (mineral) is needed for pancreatic function and tissue elasticity. A selenium deficiency is linked to cancer and heart disease (Kuo et.al., 2002). Selenium exists in both organic and inorganic forms. Many studies have shown that the most absorbable form of organic selenium is L-Selenomethionine. It is not only more absorbable but remains in the body longer, giving it a greater opportunity to be utilized by the body.

Materials and Methods
A total of 700 freshly diagnosed cancer patients (8 different cancer types) and 300 control subjects (healthy individual) were examined in this study. 10ml each of their blood samples was collected with the help of medical personnel in the University Teaching Hospitals and Federal Medical Centers in SouthWestern Nigeria. Intravenous blood (5ml) was carefully collected from the subjects into labelled heparinised bottles. The blood sample was immediately centrifuged at 10,000 revolution per minute (10,000rpm) for ten (10) minutes and separated to obtain plasma. The samples were then stored at 4°C prior to analysis. Plasma levels of the elements (Fe, Zn, K, Mg, Se, Ca, Pd, Cu, Mn and Cd) were determined using atomic absorption spectrophotometry while Na and K, were analysed using flame emission photometry as described by AOAC 1990. Statistical analysis was done using Duncan Multiple Range Test and ANOVA.

RESULTS

Results were expressed as means ± standard deviation. Graphs of same parameter with different data labels indicate significant difference at (P < 0.05).

**Figure 1:** Variation in the levels of element (sodium) within the cancer types and with control subjects.
Results were expressed as means ± standard deviation. Graphs of same parameter with different data labels indicate significant difference at (P < 0.05).

**Figure 2:** Variation in the levels of elements (Fe, Zn, Se, Cu and K) within the cancer types and with control subjects.

Results were expressed as means ± standard deviation. Graphs of same parameter with different data labels indicate significant difference at (P < 0.05).

**Figure 3:** Variation in the levels of element (Magnesium) within the cancer types and with control subjects.
Results were expressed as means ± standard deviation. Graphs of same parameter with different data labels indicate significant difference at (P < 0.05).

**Figure 4**: Variation in the levels of element (Manganese) within the cancer types and with control subjects.

Results were expressed as means ± standard deviation. Graphs of same parameter with different data labels indicate significant difference at (P < 0.05).

**Figure 5**: Variation in the levels of element (Calcium) within the cancer types and with control subjects.
Results were expressed as means ± standard deviation. Graphs of same parameter with different data labels indicate significant difference at (P < 0.05).

**Figure 6**: Variation in the levels of plasma elements (Lead and Cadmium) within the cancer types and with control subjects.

Figures 1-6 compares the plasma levels of elements in patients with different types of cancer. The results revealed no significant difference (P < 0.05) in the plasma levels of Na (Figure 1) in breast, leukemia and ovarian (135.87 ± 7.07, 138.81 ± 3.58 and 135.81 ± 3.51) cancer patients respectively while a significant increase (P < 0.05) was recorded in liver, colon, uterine, cervical and prostate (146.78 ± 4.57, 149.13±4.17, 148.25 ± 2.37, 148.35 ± 3/37 and 150.37 ± 1.43) cancer patients respectively when compared with the control subjects (138.54 ± 0.02). Similarly, Kultigin et. al., 2009 reported no significant difference (p< 0.05) in the Na level of lung cancer patients. The incidence of hypernatremia (increase in plasma Na concentration) had been reported though by Ashish et. al.,2006 in some types of cancer including prostate, cervical, liver and oesophageal cancer. However, hypernatremia in these types of cancer could be attributed to pure water loss without the signs of hypovolaemia (free water loss exceeding sodium loss) such as poor skin tugor, orthostatic hypotension, and dry mucous membranes because most of the water is lost from intracellular space which can result from extra-renal or renal causes.

Potassium imbalances are potentially life threatening. A wide variety of clinical conditions have been associated with aberrant potassium metabolism particularly, patients with cancer have been found to be at higher risk (Beverly et. al., 2005). From this study, K (fig.2) was observed to show no significant difference (P < 0.05) in breast, ovarian and colon (4.68 ± 0.44, 5.00 ± 0.73 and 4.88 ± 0.21) cancer patients respectively when compared to the control subjects (4.78 ± 0.30). The trend reported for the value of potassium in this study compared favourably with the trend reported by Kultigin et. al.,(2009) for cancer patients. A significantly raised (P < 0.05) content of potassium recorded in prostate, liver, leukemia and cervical (5.56 ± 0.51, 5.27± 0.24 4.88 ±0.24, 4.68± 0.44,) cancer patients when compared with the control subjects (4.78 ± 0.03) and among the cancer patients in this study has also been reported by Fred Schubert (2013). This result may be attributed to the breakdown of tumor cells that release potassium from the cell into the blood.

Zinc plays an important role in cellular physiology and functions as a specific activator of many enzymatic reactions (Hambidge, 1989). This study shows a decrease (P < 0.05) in the mean plasma levels of Zn (Figure 2) in all the cancer patients except in breast cancer patients where there was no significant difference (P < 0.05) when compared with the control subjects. Zinc deficiency has been implicated in the development and progression in some cancers (Palmela et. al., 2010). The general trend towards a decrease in Zn concentration in the cancer patients as demonstrated in this study, is in agreement with the results observed in cancerous liver
tissue by Kew and Mallet 1974. However, among the cancer types, elevated level of Zn was recorded in breast cancer patients (6.40 ± 0.01 mmol/L) while the least value was found in leukemia patients (3.2 ± 0.11 mmol/L). The decrease in mean plasma zinc levels observed in the cancer patients in this study may be as a result of inadequate dietary zinc intake as well as the action of leucocytes in the redistribution of the body zinc from the serum to the liver during inflammation (Cunzhi et al., 2003) and its involvement in the antioxidant defense system since the patients might be under higher oxidative stress. Zn has been proved to be an antioxidant or free-radical scavenger. It is an essential cofactor in a variety of enzymes and has antioxidant-like properties. Therefore, it can stabilize macromolecules against radical induced oxidation in vitro as well as limit excess radical production. Changes in Zn level in the blood of cancerous patients may be the cause of malignant tumor occurrence (Milde et al., 2004).

Iron, apart from its presence in all body cells, is the most abundant transition metal found in several tissues. It is involved in many processes including DNA, RNA and protein synthesis (Paralta et al., 1994). Results obtained from this study showed that the mean plasma iron level (µmol/L) was not significantly different (P < 0.05) in breast, liver, colon, uterine and prostate (9.03 ± 0.04 µmol/L, 13.22 ± 0.02 µmol/L, 11.21 ± 0.03, 8.00 ± 0.02 cancer patients cancer patients µmol/L and 10.10 ± 0.04 µmol/L) cancer patients respectively; the mean value reduced significantly (P < 0.05) in ovarian and leukemia (5.21 ± 0.05 µmol/L, and 3.02 ± 0.01 µmol/L) cancer patients respectively while a significant increase (p<0.05) was recorded in cervical (17.02 ± 0.15 µmol/L) cancer patients when compared with the control subjects (11.01 ± 0.03 µmol/L) and among the cancer patients (Figure 2). Similar observation had been reported by Nora et al., (2010). The significant decrease (P < 0.05) observed in Fe levels in some cancer patients (ovarian, uterine and leukemia) in this study, may be attributed to progression in the growth of cancer (Robert et al., 1994). The significant increase observed could be related to clinical anaemia and haematological abnormalities which were reported in some cancer cases resulting in the release of Fe from its store thus the increase of Fe in these cancer patients (Boehm et al., 2007). However, these iron metabolism molecules may be an indicator of rates of cell proliferation or may influence the growth of cancer cells. (Robert et al, 1994).

In this study, increase in the levels of plasma Cu (Figure 2) observed in prostate cancer patients could result from increased liver production of Cu as an inflammatory response in the tumor while decrease observed in other cancer types except in liver and cervical cancer patients may be due to the uptake of Cu from the blood by the cancer cells in promoting angiogenesis. However, this is in agreement with the report of Gupta et al., 1993.

Normal cells are usually protected by antioxidant enzymes from the toxic effects of high concentrations of reactive oxygen species generated during cellular metabolism while cancer cells have been found to generate reactive oxygen species (ROS) (Milde, 2004). Selenium came into general knowledge as strongly toxic, but now it is considered as an essential element with several important roles in the human body. For example, it functions as an antioxidant with interrelationship with vitamin E, and component of the antioxidant enzyme glutathione peroxidase and as such been involved in defense against oxidative stress (Carmia, 2004). Even though, it has been reported that selenium levels are low in most human cancers (Oberley 1997), this study has also supported this assertion because significant decrease (P < 0.05) in the levels of the plasma selenium levels (Figure 2) was recorded in all the cancer patients, when compared with the control subjects. However, the association between Se and cancer was strongest in prostate cancer as it has the lowest value in this type of cancer. Similar observation had been reported by Navarro-Alacron and Lopez (2000). Low concentration of Se in the plasma may be caused by the low Se content in food stuff, which mostly contributes to the intake of Se by human (Cunzhi et al., 2003). The anticancer effect of Se has been supported by animal experiments, human epidemiological investigation and intervention trials (Spevackovavi and Knoekova 1998). Yet the mechanism of Se as an anticancer agent is not clear (Navarro-Alacron and Lopez 2000).

The plasma levels of Mg and Mn were observed to be significantly different (P < 0.05) from the values obtained in the control subjects in all the cancer types (figures 3 and 4). The values however showed a significant decrease (p<0.05) when compared to the control. The decrease observed in the levels of these elements could be attributed to the fact that these elements are consumed in some metabolic pathways or replaced with carcinogenic metals in the occurrence and growth of cancer. This is in agreement with the work of Parsons and his colleagues (1974) who reported the regression of malignant tumors with hypo-magnesemia. Mg deficiency has been found to paradoxically increase the risk of, or protect against oncogenesis (Cortes and Moses, 2007). It has been proposed that Mg is central in the cell cycle, and that its deficiency is an important conditioner in precancerous cell transformation (Cortes and Moses, 2007). Also low Mn in cancer patients may be due to its consumption by cancerous cells during the development of the cancer cells (Arinola and Charles Davies 2008).

Figure 5 reveals increase in the plasma Ca levels of liver, colon, prostate and cervical cancer patients when compared to the control. The increase observed is in agreement with the work of Forte et al., 2005 and this could be attributed to progression in the growth of the cancer cells which might lead to the leakage of cancer from the bones into the blood stream or may be due to the inability of the kidneys to remove excess calcium.
from the blood.

Lead and cadmium are non-essential toxic metals that have affinity for free sulphohydryl active site of enzymes and proteins. They are known to be human carcinogens based on findings of increased risk to lung cancer and pancreatic cancer among exposed workers (Jomova and Valko 2011). Reports have shown that selenium interacts with Pb and Cd in vivo. These interactions are part of natural metal detoxification process which could result in the metabolic inactivation of Se, however, at high exposure levels, Pb and Cd may overtime produce a state akin to Se deficiency thereby aborting the cancer protecting effects of Se (Alatise, 2010). This study however shows a significant increase (P < 0.05) in the plasma levels of Pb and Cd (Figure 6) in all the cancer types when compared to the control subjects and a significant increase was observed in liver cancer patients among other cancer patients. The elevated levels recorded in these metals could be attributed to their involvement in the generation of reactive oxygen species which could result in lipid peroxidation, DNA damage and altered gene expression (Kayode et al., 2014), hence in the pathogenesis of cancer (Harouny, 2010).

Conclusion
The pattern of alteration in the levels of elements of the cancer patients observed in this study suggests that deficiency of elements may be a risk factor in the occurrence and progression of cancer and its complication.

REFERENCES


