Journal of Advances in Medicine and Medical Research

28(2): 1-7, 2018; Article no.JAMMR.44891
ISSN: 2456-8899
(Past name: British Journal of Medicine and Medical Research, Past ISSN: 2231-0614, NLM ID: 101570965)

Evaluation of Vitamin E and Selenium Levels in Breast Cancer Patients in Port Harcourt Metropolis, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author AEB designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Author IE managed the analyses of the study. Authors AEB and IE managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMMR/2018/44891
Editor(s):
(1) Dr. Evangelos Marinos, Associate Professor, University of Athens, School of Medicine, Laboratory of Biology, Athens, Greece.
Reviewers:
(1) Prashant Sharma, Seoul National University, South Korea.
(2) V. O. Imieje, University of Benin, Nigeria.
(3) Naoki Hashimoto, Kindai University, Japan.
Complete Peer review History: http://www.sciencedomain.org/review-history/27400

Original Research Article

Received 11 September 2018
Accepted 17 November 2018
Published 24 November 2018

ABSTRACT

Breast cancer has become the most common cancer diagnosed in women and reason for most death arising from cancer, especially in developing countries. In Port Harcourt, Nigeria, there has been a steady increase in the incidence of breast cancer, surpassing cervical cancer. Hence this study is to find the link between micronutrients and the risk of breast cancer as research studies are looking towards possible ways the human body resistance could be adequately improved or enhanced to help the body overcome the onslaught of most cancer-causing agent humans are exposed to. Serum and urine selenium levels and serum vitamin E levels in 100 breast cancer patients and 50 normal healthy individuals were investigated using the ELISA method. Graphpad prism (version 7) was used for statistical analysis. The respective mean values for serum and urine selenium (µg/l) and serum vitamin E (µg/ml) were reduced in the cancer subjects 0.89 ± 1.0; 0.19 ±0.03 and 11.44 ± 6.1 when compared with control subjects with values of 4.92 ± 3.2; 4.46 ± 3.9

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and 17.68 ± 16.0 for serum and urine selenium and serum vitamin E samples respectively. The significantly ($p<0.05$) lowered values in the cancer patients could be indicating that reduced levels of selenium and /or vitamin E are the possible risk of breast cancer.

Keywords: Antioxidants; selenium; vitamin E; breast cancer.

1. INTRODUCTION

Breast cancer, the most diagnosed malignancy (tumour on neoplasm) in women, making up about 25% of all cancers, is now the leading cause of mortality in the world over, which is about 15% of all cancers deaths [1,2]. More than half of the women diagnosed with cancer of the breast world over are not due to known risk factors for the disease. A large body of research indicates that toxic chemicals are likely to raise the risk of developing breast cancer as known risk factors is responsible for around 5 – 10% of cancer of the breast [3,4,5]. Many scientists and researchers stated also that the burden of environmentally induced cancer has been grossly underestimated with people living both in the developing world and developed world, having to deal continually with a different combination of this dangerous exposure. These chemicals or toxins increase the risk of cancer by free radicals, which act on biochemical systems during oxidative processes [3,6].

Free radicals, highly reactive chemicals that have the ability to destroy cells are produced during oxidative processes when an atom or a molecule either gains or losses an electron. Free radicals are formed naturally or as a consequence of external factors in the body and play vital roles in several normal cellular processes [7,8,9]. At an increased level, however, free radicals can be dangerous to the body components of cells, such as DNA, proteins, and cell membranes. The alteration to cells caused by free radicals, especially the damage to DNA, could play a role in the growth of cancer and other health conditions [10,11,12,13]. Unusually increased level of free radicals in the body can be due to subjection to ionising radiation and other environmental toxins. When ionising radiation attacks an atom or a molecule in a cell, an electron may be lost, leading to the production of a free radical. The generation of an unusually increased level of free radicals is the process by which ionising radiation eliminates cells. However, some cancer causing toxins, such as cigarette smoke, some metals, and high-oxygen atmospheres, may have large amounts of free radicals or stimulate the body's cells to produce more free radicals [1,14,15]. Free radicals with the element oxygen, otherwise known as reactive oxygen species, (ROS) are the most common type of free radicals produced in living tissue [8]. These free radical effects are counteracted by antioxidant defence mechanism.

Antioxidants are chemicals that interface with and counteract free radicals, preventing them from body destruction [8]. The body makes some of the antioxidants it uses referred to as endogenous antioxidants. Nonetheless, the body depends on exogenous sources, principally, through the diet. These exogenous antioxidants are basically called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants. Some dietary antioxidants are also available as dietary supplements [16]. Dietary antioxidants include beta-carotene, lycopene, and vitamins A, C, and E (alpha-tocopherol). The mineral, selenium is believed to be a dietary antioxidant, through its antioxidant effects are most probably due to the antioxidant activity of proteins that have this element as an essential component (i.e., selenium-containing proteins), and not to selenium itself [16,17,18].

Research studies keen in the prevention of cancer are looking towards possible ways the human body resistance could be adequately improved or enhanced to help the body overcome the onslaught of most cancer causing agent humans are exposed to. Emphasis is on nutritional therapy as this could be the simplest and perhaps the new most effective way for the prevention of cancer and other degenerative diseases [19]. Antioxidants are believed to play this role.

Antioxidant slows, averts or moves back oxidative destruction to target molecule by acting on different procedures in the oxidative protocol [18]. The human body complex antioxidant defence system constitutes dietary intakes of antioxidants in the form of fruits and vegetables and endogenous production of antioxidant compounds e.g. glutathione. Antioxidants are grouped into enzymatic and non-enzymatic strategies. The enzymatic strategies include superoxide dismutase (SOD), catalase,

glutathione peroxidase and glutathione reductase while non enzymatic strategies involve vitamins A, C and E; lipoic acid, mixed carotenoid, glutathione, various bioflavonoids, minerals such as copper, zinc, manganese, and selenium etc. They could function singly or in combination against diverse free radicals. Vitamin E prevents the multiplication of lipid peroxidation, the association of vitamin C and E impedes the development of hydroperoxides and metals complexing antioxidants such as penicillamine, inhibit free radicals propagation in lipid peroxidation [20,4,21,22].

Selenium, an important trace mineral sourced from food is required in small amounts and as a constituent of several enzymes, is vital for sustaining good health. Glutathione peroxidase (GSH-Px), selenium-containing enzyme catalyses the reduction of hydrogen peroxides and organic hydro peroxides, hence, defending the cell membrane and the immune system from oxidative damage [10,18]. Selenium deficiency such as in exhaustion, high cholesterol, liver impairment and infections leads to a serious problem and as a component of various selenoproteins is involved in important roles in reproduction, thyroid hormone metabolism, DNA synthesis and protection from oxidative damage and infection [23]. Selenium is seen in different forms and at different absorption levels, used in union with countless divergent proteins in the body. The three forms of selenium, each with its set of distinctive abilities and vital roles in cancer prevention are sodium selenite, L-selenomethionine, and selenium-methyl L-selenocysteine [24].

The possible process where selenium is involved in cancer prevention in humans is in relation to the antioxidant outcomes via GSH-Px. Selenium helps to control and defend the body from the damaging effects of free radicals [9]. Some breakdown products of selenium are known to stop or slow down tumour growth by strengthening the immune system cell functions and repressing the development of blood vessels to the tumour [15].

A research study by [20], concluded that selenium and vitamin E was not beneficial to the fight against cancer. When the results were reassessed, the researchers found that synthetic alpha-tocopherol was the selected vitamin, which could pull gamma tocopherol out of cells, increasing the cancer cells risk.

Vitamin E; a general term used randomly to relate a group of 8 similar but distinctive naturally occurring compounds termed tocopherols and tocotrienols and synthetic vitamin E (a chemical mixture of 12.5% authentic RRR-$\propto$-tocopherol and 87.5% stereoisomers) [22]. The natural vitamin E RRR-$\propto$-tocopherol or δ-$\propto$-tocopherol and synthetic vitamin E, all-racemic-$\propto$-tocopherol or di-$\propto$-tocopherol are not chemically equivalent. RRR-$\propto$-tocopherol, the most prevalent form in the human body is known as a free radical scavenging antioxidant, vital for controlling polyunsaturated fats from peroxidation and its deficiency alters mammalian fertility [25,19].

Cancers are clonal in origin, from normal cells, and go through several genetic alterations. Nonetheless, the huge complexities in the conversion of normal cells to cancer cells, selection for survival among cancers seem to be a common event [11]. Changing cancer cells do not follow a pattern but involves a stage-by-stage process, preferring genetic modifications that extend to cell survival [7]. Developed cancers that acquire growth factor separation and resistance to apoptosis exhibit more survival edge [22]. Laboratory studies have shown that possession of resistance to apoptosis by breast, ovarian and prostate cancer cells are reversible events, with vitamin E compounds, eliciting cancer cells to undergo cell death by repairing pro-death signalling pathways [26,27]. Various studies indicate that premenopausal women with very low intake of Vitamin E are 2 times more likely to get breast cancer [21]. Also, studies on total Vitamin E intake indicate that women who have a family history of breast cancer could get an 80% risk reduction against 60% for women with no family history of cancer [22]. However, the reduction in risk is inconsistent amongst different women groups such as pre and postmenopausal, with and without a family history of breast cancer.

Research studies have shown recently that metals act as facilitators in the oxidative determination of biological macromolecules and metals elicit reactive oxygen species (ROS), giving off free radicals as a consequence [25]. The accumulation of ROS could affect epigenetic factors leading to its modification. There is a delicate balance between ROS and RNS production and removal by antioxidants. The values are lower in breast cancer when these antioxidants are overwhelmed by the amount of ROS and RNS generated.

2. MATERIALS AND METHODS

2.1 Study Population

The study population of 150 subjects, 100 subjects, who were serologically and histologically confirmed as positive for breast cancer of either ductal carcinoma, ductal carcinoma in situ and/or lobular carcinoma served as the test subjects. Molecular subtypes were not confirmed. These females, which attended the Surgery clinics of University of Port Harcourt Teaching Hospital, Braithwaite Memorial specialist Hospital and Meridrien Hospital all in Port Harcourt with a palpable lump in the breasts, were selected for the study. 50 patients, who served as control, were individuals with no known individual or family history of either breast or any other type of cancer excluded by thorough physical examination.

Informed consent was obtained from each subject. Ethical clearance (UPTH/ADM/90/S.II/VOL.X/775) was also obtained from the ethics committee of the various institutions before the commencement of the study. The subjects were all recruited and implicated at the pre-surgical stage at the different treatment centres.

2.2 Examination of the Patients

A demographic 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 any kind of food supplement prior to diagnosis, use of tobacco as well as alcohol among other things.

2.3 Collection of Samples

Samples were collected from the subjects at the treatment centres. Blood samples were collected in plain sample bottles while urine samples were collected in sterile urine sample bottles.

2.4 Experimental Design

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

2.5 Sample Treatment

Serum was separated from whole blood and kept frozen in the refrigerator at a temperature of -2 to -8ºC until ready to use. Urine was preserved prior to analysis.

2.6 Sample Analysis

Serum and urine selenium was determined using a solar thermo-elemental flame atomic absorption spectrometer (FAAS) (Model S4-71096) American standard. The serum and urine samples were first treated by digestion with nitric, perchloric and hydrochloric acids. The digested sample was assayed for heavy metals with FAAS with appropriate wavelengths and hollow cathode lamps. Vitamin E was analysed (using the principle of ELISA method [24]) using stat fax plate reader.

2.7 Statistical Analysis

Graphpad prism version 7 was used for the statistical analysis. Results obtained were reported as Mean±Standard deviation. Students' statistical t-test was used for the comparative analysis. Statistical significance was determined at $p<0.05$.

3. RESULTS

In this study, the assessment of vitamin E and selenium in breast cancer patients was carried out. When the mean levels of control (apparently healthy individuals) were compared with breast cancer patients, the result obtained shows control had 4.92 ± 3.2, 4.46 ± 3.9 and 17.68 ± 16.0 for serum selenium, urine selenium and serum Vitamin E levels respectively while the breast cancer patients had 0.89 ± 1.0, 0.19 ± 0.03 and 11.44 ± 6.1 for serum selenium, urine selenium and serum Vitamin E levels respectively. The comparative analysis of the serum selenium, urine selenium and serum vitamin E in control subjects against the breast cancer patients indicated significant ($p<0.05$) reduction in the concentration of vitamin and selenium in urine and serum were observed in the breast cancer patients (Table 1, Fig. 1).

4. DISCUSSION

From the results obtained the mean levels of selenium are significantly higher ($p<0.05$) in the control samples than in the breast cancer patients in both body fluids (serum and urine)

Table 1. Mean Levels of selenium in serum and urine and serum vitamin E of test and control subjects

Parameters	Serum Se (µg/l)	Urine Se (µg/l)	Serum Vit. E (µg/ml)
Test	0.89 ± 1.0	0.19 ± 0.03	11.44 ± 6.1
Control	4.92 ± 3.2	4.46 ± 3.9	17.68 ± 16.0
Tvalue	11.45	10.88	3.0187
Pvalue	<0.05	<0.05	0.0016
Remarks	**S**	**S**	**S**

S = indicates significant level

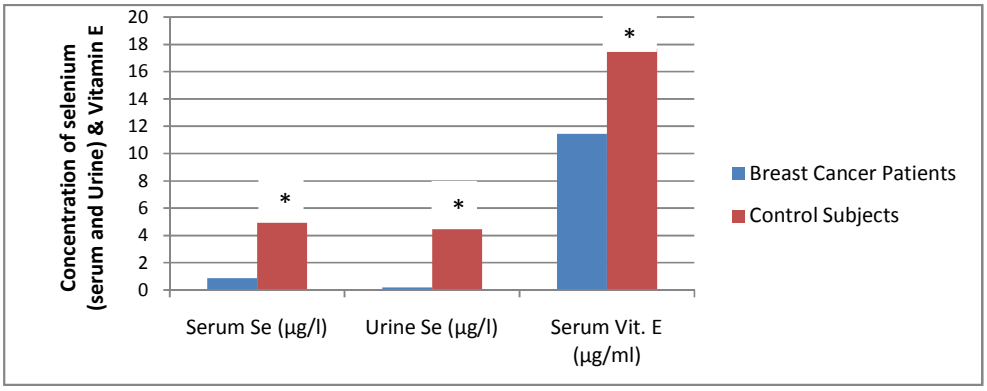

Fig. 1. Graph of Selenium (serum and Urine) and Vitamin E (serum) of Breast Cancer Patients and Control (healthy) subjects

**Significantly higher values of selenium and Vitamin E*

assayed. Selenium is an essential trace metal needed in minute quantity for human nutrition mostly identified as a cofactor implicated in important antioxidant enzymes (selenoproteins) involved in the reduction of oxidative principles, which could lead to premature ageing and chronic diseases [28,29,30]. Selenium has long been known to aid in several different types of degenerative diseases [10] but in recent times, there is an improved research interest in its main role in anti cancer process [26]. This is because studies have evidenced favourable support that selenium activates expression of the gene to bring down initiation of a tumour, differentiation and spread. Epidemiological studies have shown that communities with reduced levels of selenium are at significantly elevated risk of contracting several forms of cancer [31], with reduced levels of selenium in different body fluids and tissues been associated with a 2-3 fold increase in general cancer risk [23].

Selenium is present in three main different forms needed for cancer prevention and each giving a significant window of cancer preventive outcomes. Selenium acts by several complementary pathways to control cancers from growing (pleiotrophy). Pleiotrophy enables researchers to inactivate cancer effects on many different angles and at different stages. Selenium regulates possible cancer cells from gaining complete tumour status by regulation of lipoxygenesis (enzymes that create inflammation), reduction of oxidative stress, inactivation of molecular transcription factors, shutting down of the essential cell replication cycle, improvement of immune system activity, activation of apoptosis and limiting outcomes on tumour spread. Debatable results have been reported concerning the role of selenium in carcinogenesis. Depending on selenium distribution geographically and variations in cancer incidence, different studies speculate that reduction in the level of selenium could be a risk factor for cancer in some parts of the world [14] and some clinical trials showed an inverse link between selenium levels and death from cancer [25]. Another clinical study revealed a positive link between selenium levels and cancer. Selenium methyl L-selenocysteine boosts tamoxifens's potential to suppress the proliferation of breast cancers in a mammary tumour elicited in laboratory mice. This breakthrough has led to the production of selenium methyl L-selenocysteine for chemoprevention in breast cancer [6].

The table, above also represents the values of vitamin E in serum samples of both breast cancer and control patients. The result also showed that vitamin E levels are significantly ($p<0.005$) higher in the control samples than in the test. Pan et al. [30], in their study stated that vitamin E intake for a sustained period of time had a protective outcome in postmenopausal women by significantly lowering risk of breast cancer. Oxidative stress and lipid peroxidation processes are established sources of breast cancer scavenging free radicals, eliciting cancer cell death and controlling cancer cell growth is the function of antioxidants [25,19]. Nonetheless, physiological menopausal status which presents important changes in women such as body fat distribution, bone and breast density, ovarian and endometrial alterations as well as cardiovascular changes among others could influence growth of cancer and/or association with antioxidants and breast tissue. Vitamin E acts by controlling the generation of free radicals as peroxyl radical scavenger and also by regulating polyunsaturated fatty acids in phospholipids membrane and plasma lipoproteins [17,12,2].

Certain research studies on supplementation of vitamin E shows that women with a family history of breast cancer could have lowered risk as much as 80% against 60% for women without family history of with variation among different women groups [11]. Also, two extensive reviews on the association between dietary intake of vitamin E and α-tocopherols levels in subcutaneous adipose tissue concluded that vitamin E from dietary sources could moderately have a protective outcome on breast cancer [21].

5. CONCLUSION

The major function of selenium is in the control of oxidation of fats as a constituent of glutathione peroxidase enzyme. In the combination with vitamin E, immune function is maintained, leading to fight against free radicals generated through oxidative processes and lipid peroxidation, degenerative diseases, cancer included as a consequence.

6. RECOMMENDATION

From the above therefore, it is pertinent to look towards effective nutritional therapy in the fight against degenerative diseases.

CONSENT

Informed consent was sort from the patients and only those who gave consent participated in the study, a structured questionnaire on demographic data was administered to all participants.

ETHICAL APPROVAL

Ethical clearance was obtained from the Rivers State Hospitals Management Board.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/27400