ANTIOXIDANT STATUS OF BREAST, CERVICAL AND OVARIAN CANCER PATIENTS AT VARIOUS MENOPAUSAL STAGES IN LAGOS STATE, NIGERIA

ABSTRACT

Cancer is a collection of diseases which involves the abnormal growth of cells with the potential to invade or spread to other parts of the body. The aim of this study is to access the antioxidant status of women with female predominant cancer (breast, cervical and ovarian) in relationship with their menopausal stages. Blood samples were collected from 180 freshly diagnosed female patients of breast, cervical and ovarian cancer at Lagos State University Teaching Hospital, Idi- Araba, Mushin, Lagos and Lagos State University Teaching Hospital Ikeja, Lagos and 60 relatively healthy subjects at different menopausal stages. Serum catalase (CAT), Superoxide dismutase (SOD) activites, Reduced Glutathione (GSH), Vitamin C and E concentrations were evaluated in subject's blood sample using standard established methods. The results obtained were subjected to statistical analysis ($p < 0.05$). However, the results of the female cancer patients at each menopausal stage were compared to premenopausal, menopausal and postmenopausal control groups, while the results obtained from the menopausal and postmenopausal control subjects were compared to the premenopausal control subjects. There was significant decrease ($p < 0.05$) in the activities of CAT, SOD and in the concentrations of GSH, Vitamins C and E in all the menopausal stages when compared to the control groups. However, MDA concentrations showed significant increase ($p < 0.05$) in all the menopausal stages in comparison to the corresponding control groups. These findings suggest that cancer patients might be at risk from oxidative cell damage. Therefore, further research is required in this field with a view of improving the management of cancers predominant in females.

1. INTRODUCTION

Cancer is a collection of diseases which involves the abnormal growth of cells with the potential to invade or spread to other parts of the body [1]. They form a subset of neoplasms. A neoplasm or tumor is a group of cells that have undergone unregulated growth and will often form a mass or lump, but may be distributed diffusely [1]. The most common types are breast cancer, colorectal cancer, lung cancer, cervical cancer, liver cancer, stomach cancer and skin cancer [2; 3].

Deaths from cancer have been reported increasing primarily due to longer lifespans and lifestyle changes in the developing world [4]. The most significant risk factor for developing cancer is age [5]. Although it is possible for cancer to strike at any age, most patients with invasive cancer are over 65 [5]. Cancer deaths in women are linked to obesity, poor diet, lack of physical activity, and excessive drinking of alcohol [1; 6]. Other factors include certain infections, exposure to ionizing radiation and environmental pollutants [7]. In the developing world about 20% of cancers are caused by infections which includes hepatitis B, hepatitis C and human papillomavirus infection [1].These factors act, at least partly, by changing the genes of a cell. Subsequently, genetic mutations result in the development of cancer. Approximately 5–10% of cancers are due to inherited genetic defects from a person's parents [1].

Menopause is a progressive development of reproductive aging which affects a sequence of hormonal changes over several years leading to cessation of ovarian follicular activity and menstrual cycles in women. There are three major menopausal stages, they are: premenopause, perimenopause and postmenopause. Premenopause is the years leading up to the last period, when the levels of reproductive hormones are becoming more variable and lower, and the effects of hormone withdrawal are present [8]. Perimenopause means around the menopause. This refers to the menopause transition years, a time before and after the date of the final episode of flow while, postmenopausal describes women who have not experienced any menstrual flow for a minimum of 12 months, with an exception of being pregnant or lactating [8].

There could be a possibility that menopausal stages have a role to play in cancer owing to increase in the occurrence of cancer among women and its uncertain relationship with menopause. Therefore, assessing the relationship between menopausal stages and metabolic

2

changes in cancer women might go a long way in the management of female cancers. The aim of this study is to access the Antioxidant status of women with female related cancer (breast, cervical and ovarian) in relationship with their menopausal stages.

2. MATERIALS AND METHODS

2.1. PARTICIPANTS

The subjects were one hundred and eighty women freshly diagnosed of breast, cervical and ovarian cancer patients at Lagos University Teaching Hospital, Idi- Araba, Mushin, Lagos and Lagos State University Teaching Hospital Ikeja, Lagos while sixty apparently healthy women and nurses served as control subjects. All of the participants belonged to the same age range of 25 to 65 years.

2.2. EXCLUSION Criteria

Exclusion criteria included women with pregnancy, amenorrhoea and any previous history of chronic and metabolic diseases.

2.3. DATA COLLECTION

The ethical clearance was sought from Lagos University Teaching Hospital Health Research Ethics Committee with protocol number ADM/DCST/HREC/APP/1337. Informed consent was obtained from the participants (subjects and control) after the study guidelines had been explained to them before clinical history was taken and anthropometric indices such as age, menopause year using structured questionnaire.

2.4. SAMPLE COLLECTION

Venous blood samples were collected from each subject and distributed into non anticoagulated bottles. The samples were spun using bucket centrifuge at 4000rpm for 3minutes and the supernatant (serum) was collected and analysed for biochemical profile of blood

2.5. SUBJECTS GROUPING

The subjects were grouped as follows:

Group 1:   20 premenopausal women with breast cancer

Group 2:   20 menopausal women with breast cancer

Group 3:   20 postmenopausal women with breast cancer

Group 4:   20 premenopausal women with cervical cancer

Group 5:   20 menopausal women with cervical cancer

Group 6:   20 postmenopausal women with cervical cancer

Group 7:   20 premenopausal women with ovarian cancer

Group 8:   20 menopausal women with ovarian cancer

Group 9:   20 postmenopausal women with ovarian cancer

Group 10:   20 healthy premenopausal women

Group 11:   20 healthy menopausal women

Group 12:   20 healthy postmenopausal women

The age range (in years) of the premenopause, menopause and postmenopause groups were between 25-40, 42-46 and 50-65 respectively.

2.6. BIOCHEMICAL ANALYSIS

2.6.1. Determination of Lipid Peroxidation (MDA)

Lipid peroxidation (Malondialdehyde) was estimated as evidenced by the formation of thiobarbituric acid reaction substances (TBARS) according to the method of Ohkawa *et al*. (1979) [9].

2.6.2. Estimation of Superoxide Dismutase

The Superoxide dismutase (SOD) activity was determined spectrophotometrically using the method of Marklund and Marklund (1974) [10].

2.6.3. Estimation of Serum Reduced Glutathione (GSH) Concentration.

Reduced glutathione (GSH) cocentration was measured according to the method of Beutler and Kelly (1963) [11].

2.6.4. Determination of Catalase Activity

Catalase activity was determined spectrophotometrically as the rate of disappearance of hydrogen peroxide according to the methods of Beers and Sizer (1952) [12].

2.6.5. Determination of Vitamin C (Ascorbic Acid)

Ascorbic acid was estimated using the method of Omaye *et al*. (1979) [13].

2.6.6. Determination of Alpha –Tococpherol (Vitamin-E)

Vitamin E was determined spectrophotometrically using the method of Rutkowski and Grzegorczyk, (2007) [14].

4

The data collected from the results was analyzed using one - way Analysis of Variance (ANOVA) followed by post-hoc Duncan test, and expressed as mean ± SEM (standard error of mean) with P value less than 0.05 ($p<0.05$) considered to be statistically significant. The test for statistical significance was carried out at 95% confidence limit. The analysis was done using Statistical Package for Social Sciences (SPSS), version 22.

3. RESULTS

There was a significant decrease ($p<0.05$) in the serum catalase activity of all the cancer patients when compared to the control subjects across all the menopausal stages. The catalase activity of postmenopausal control group showed significant decrease ($p<0.05$) while the menopausal control group showed no significant difference when compared to the premenopausal control group (Table 1).

The SOD activity in the cancer cases were significantly decreased ($p<0.05$) when compared to the control subjects in all the menopausal stages. The postmenopausal control subject showed a significant decrease ($p<0.05$) in SOD activity when compared to the Premenopausal control subjects (Table 2).

Also, there was significant decrease ($p<0.05$) in the GSH level in all the menopausal stages of all the cancer patients when compared to the control subjects. Also, the menopausal and postmenopausal control subjects showed a significant decrease in GSH levels when compared to the premenopausal control subjects (Table 3).

The level of MDA was significantly increased ($p<0.05$) in the MDA levels of all the cancer patients when compared to the corresponding control subjects while the menopausal and postmenopausal control subjects showed a significant increase in MDA levels when compared to the Premenopausal control subjects (Table 4).

Vitamin C and E levels of all the cancer patients were significantly decreased ($p<0.05$) when compared to the control subjects in all the menopausal stages. Also, the menopausal and postmenopausal control subjects showed a significant decrease ($p<0.05$) in the vitamin C levels when compared to the Premenopausal control subjects (Table 5), while only postmenopausal stage of control subject showed significant decrease in Vitamin E levels when compared to the Premenopausal control subjects (Table 6).

Table 1: Serum catalase activity (unit/mgprotein) of control, breast, cervical and ovarian cancer patients at various menopausal stages.

Menopausal Stages	Control	Breast Cancer	Cervical Cancer	Ovarian Cancer
Premenopause	37.03 ± 3.66	$24.43 \pm 2.62^{*}$	$14.92 \pm 1.35^{*}$	$17.99 \pm 1.61^{*}$
Menopause	35.64 ± 11.36	$14.38 \pm 1.54^{*}$	$10.51 \pm 1.12^{*}$	$11.64 \pm 1.15^{*}$
Postmenopause	$23.19 \pm 1.72^{\#}$	$20.32 \pm 2.17^{*}$	$11.77 \pm 1.26^{*}$	$14.73 \pm 1.57^{*}$

Values are expressed as mean ± standard error of mean (SEM). Values marked with * are significantly different at $p < 0.05$ compared to the control subjects across all the menopausal stages, while values marked with # are statistically different control group at $p<0.05$ compared to Premenopausal control group.

Table 2: Serum SOD activity (unit/mgprotein) of control, breast, cervical and ovarian cancer patients at various menopausal stages.

Menopausal Stages	Control	Breast Cancer	Cervical Cancer	Ovarian Cancer
Premenopause	37.61 ± 4.02	$26.31 \pm 0.80^{*}$	$24.43 \pm 2.62^{*}$	$26.24 \pm 2.81^{*}$
Menopause	$24.31 \pm 2.38^{\#}$	$18.84 \pm 2.02^{*}$	$22.79 \pm 2.45^{*}$	$21.31 \pm 2.27^{*}$
Postmenopause	34.97 ± 3.75	$15.70 \pm 0.61^{*}$	$19.30 \pm 1.76^{*}$	$24.91 \pm 2.44^{*}$

Values are expressed as mean ± standard error of mean (SEM). Values marked with * are significantly different at $p < 0.05$ compared to the control subjects across all the menopausal stages, while values marked with # are statistically different control group at $p<0.05$ compared to Premenopausal control group.

Table 3: Serum GSH level (unit/mgprotein) of control, breast, cervical and ovarian cancer patients at various menopausal stages.

Menopausal Stages	Control	Breast Cancer	Cervical Cancer	Ovarian Cancer
Premenopause	16.91 ± 1.81	$10.04 \pm 0.60^*$	$11.63 \pm 1.06^*$	$9.61 \pm 1.03^*$
Menopause	$9.61 \pm 0.93^\#$	$5.00 \pm 0.54^*$	$7.70 \pm 0.84^*$	$6.21 \pm 0.66^*$
Postmenopause	$12.98 \pm 1.39^\#$	$7.59 \pm 0.38^*$	$7.30 \pm 0.78^*$	$8.34 \pm 0.78^*$

Values are expressed as mean ± standard error of mean (SEM). Values marked with * are significantly different at $p < 0.05$ compared to the control subjects across all the menopausal stages, while values marked with # are statistically different control group at $p<0.05$ compared to Premenopausal control group.

Table 4: Serum MDA level (µmol/mg) of control, breast, cervical and ovarian cancer patients at various menopausal stages.

Menopausal Stages	Control	Breast Cancer	Cervical Cancer	Ovarian Cancer
Premenopause	0.05 ± 0.001	$0.24 \pm 0.01^*$	$0.25 \pm 0.17^*$	$0.12 \pm 0.01^*$
Menopause	$0.29 \pm 0.09^\#$	$0.54 \pm 0.21^*$	$0.64 \pm 0.02^*$	$0.39 \pm 0.01^*$
Postmenopause	$0.12 \pm 0.01^\#$	$1.07 \pm 0.49^*$	$0.55 \pm 0.01^*$	$0.21 \pm 0.05^*$

Values are expressed as mean ± standard error of mean (SEM). Values marked with * are significantly different at $p < 0.05$ compared to the control subjects across all the menopausal stages, while values marked with # are statistically different control group at $p<0.05$ compared to Premenopausal control group.

Table 5: Serum Vitamin C (mg/dl) level of control, breast, cervical and ovarian cancer patients at various menopausal stages.

Menopausal Stages	Control	Breast Cancer	Cervical Cancer	Ovarian Cancer
Premenopause	1.69 ± 0.13	$1.06 \pm 0.05^*$	$0.94 \pm 0.04^*$	$1.14 \pm 1.07^*$
Menopause	$1.41 \pm 0.27^\#$	$0.96 \pm 0.04^*$	$0.73 \pm 0.02^*$	$0.99 \pm 0.06^*$

| Postmenopause | $1.19 \pm 0.11^{\#}$ | $0.59 \pm 0.05^{*}$ | $0.80 \pm 0.79^{*}$ | $1.04 \pm 0.03^{*}$ |

Values are expressed as mean ± standard error of mean (SEM). Values marked with * are significantly different at $p < 0.05$ compared to the control subjects across all the menopausal stages, while values marked with # are statistically different control group at $p<0.05$ compared to Premenopausal control group.

Table 6: Serum Vitamin E (mg/dl) level of control, breast, cervical and ovarian cancer patients at various menopausal stages.

Menopausal Stages	Control	Breast Cancer	Cervical Cancer	Ovarian Cancer
Premenopause	1.18 ± 0.01	$0.94 \pm 0.02^{*}$	$0.66 \pm 0.02^{*}$	$0.73 \pm 0.01^{*}$
Menopause	1.13 ± 0.02	$0.75 \pm 0.01^{*}$	$0.64 \pm 0.02^{*}$	$0.63 \pm 0.01^{*}$
Postmenopause	$1.03 \pm 0.04^{\#}$	$0.63 \pm 0.01^{*}$	$0.62 \pm 0.03^{*}$	$0.60 \pm 0.02^{*}$

Values are expressed as mean ± standard error of mean (SEM). Values marked with * are significantly different at $p < 0.05$ compared to the control subjects across all the menopausal stages, while values marked with # are statistically different control group at $p<0.05$ compared to Premenopausal control group.

4. DISCUSSION

The result showed that the levels of antioxidants were significantly decreased (p<0.05) in patients suffering from cancer compared to control subjects at their corresponding menopausal stage. GSH, SOD and catalase activities were significantly decreased (p<0.05) in all cancer cases showing that production of free radicals was high. The decreased activities of SOD and CAT may be due to the increase in erythrocyte lipid peroxidation in cancer patients [15]. They also can act as anticarcinogens, and inhibitors at initiation and promotion/ transformation stage in carcinogenesis.

Vitamin E and vitamin C levels of control subjects were significantly higher (p<0.05) than the cancer cases all through the menopausal stages. Vitamin E is a universal participant of antioxidant defense reactions in biological membranes, since it acts at all steps of membrane oxidative damage, and act as a first line of defense against lipid peroxidation [16]. Decrease in vitamin E in patients with cancer could be due to the possibility that vitamin E reacts very rapidly with molecular oxygen and free radicals, the role of which has been implicated in carcinogenesis. It is suggested that vitamin E acts as a scavenger protecting polyunsaturated

8

fatty acids from peroxidation reaction in cancer. As vitamin E was found to decrease in breast cancer patients, they may not be sufficient enough to counter free radical attack, thereby resulting in oxidative stress. In the present study a positive correlation between vitamin E deficiency and lipid peroxide formation in postmenopausal women in all the cancer cases.

The serum MDA level in cancer patients was significantly higher ($p<0.05$) than the control subjects. Increased lipid peroxidation in serum and tissues has been reported in breast cancer compared to the control subjects by Gonenc *et al.* (2005) [17]. These findings suggest that cancer patients might be at risk from oxidative cell damage. The increase in the rate of lipid peroxidation causes the increased production of MDA that leaks into the blood stream, consequently causing increased levels of MDA in patients with cancer. The enhanced lipid peroxidation observed in the cancer patients may also be due to depletion of the activities of enzymes SOD and catalase which are the free radical scavenging enzymes or higher production of O_2- and H_2O_2. Hence, increased lipid peroxidation in cancer patients, in the present study, might be ascribed to the deficiency of vitamin E.

The variation in the antioxidant parameters of menopausal control groups might be due to imbalance between the oxidant species that are produced by metabolism and the effective action of the antioxidant system. Also, the imbalance might be due to progression in aging leading to decline of estrogen levels and hence cause of age-related oxidative stress [18].

5. CONCLUSION AND RECOMMENDATION

It can therefore be concluded from this study that the variations observed in the antioxidant parameters of each menopausal phase gave pivotal information on the relationship of menopause with female cancers. Also, the transition from each menopausal stage of life, oxidative stress might play a role as part of important risk factors in female cancers. Hence, more research is required in this field with a view of improving the management of cancers predominant in females.

ETHICAL APPROVAL

The ethical clearance was sought from Lagos University Teaching Hospital Health Research Ethics Committee with protocol number ADM/DCST/HREC/APP/1337.

9

DECLARATION OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this research work.

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