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ASSESSMENT OF LYMPHOCYTE DNA DAMAGE IN BREAST CANCER AND ITS ASSOCIATION WITH AGE AND MENOPAUSAL STATUS: A STUDY AMONG TAMIL WOMEN.

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ABSTRACT

Breast cancer development is intimately related to DNA damage and DNA repair failures. The aim of this study was to investigate the level of DNA damage in the lymphocytes of breast cancer patients and to find its association with age and menopausal status. This study comprises of 75 breast cancer patients, equally categorized into three groups, based on the disease stage namely, non metastatic group, metastatic group and post treatment group. Control consisted of 25 healthy subjects. Lymphocytes were isolated from blood samples and DNA damage was assessed by comet assay. DNA damage was remarkably higher in breast cancer patients than in healthy subjects. Increased DNA damage was found in patients with metastases and post-treatment group. A positive correlation has been obtained in case of age and DNA damage. DNA damage was also high in post menopausal group when compared with pre menopausal group. These findings reveal that DNA damage increases with disease progression. DNA damage was also found to be positively associated with age and menopausal status of women with breast cancer. This stronger correlation observed may be useful in elucidating the mechanism of disease pathogenesis.

KEY WORDS: Breast cancer, Metastasis, Comet assay, DNA damage, Lymphocyte

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INTRODUCTION

Breast cancer is the most common female malignancy and second to lung cancer in terms of mortality. It is the leading cause of death among women aged 40 to 50 years [1]. Breast cancer is a disease caused by a complex combination of genetic and environmental factors. Mammalian cells are constantly exposed to exogenous and endogenous DNA-damaging agents. Under these circumstances, women may develop cytogenetic alterations in oncogenes and tumour suppressor genes, leading to cellular transformation and neoplasm [2]. Higher levels of DNA damage may predispose individuals to breast cancer[3]. Age and menopausaul status are important factors in determining the risk of breast cancer. After gender, age is the single most important factor in breast cancer development. Oxidative DNA damage is an ongoing process of aging and is elevated in patients with disease [4] . Similarly for women who experience early menses and late menarche, the risk of developing breast cancer is several fold higher. Comet assay or single cell gel electrophoresis is widely used to measure and analyze DNA breakage in mammalian cells. It is one of the most sensitive and accurate technique and has been considered a valuable tool for population monitoring, for assessing the role of oxidative stress in human disease, mechanisms of mutagenesis and genotoxicology [5]. DNA damage appears to be ubiquitous in the biological world. Many studies have indicated the presence of DNA damage in breast cancer patients. However association of DNA damage with age and menopausaul status in breast cancer patients remains unclear. Hence this study was carried out to investigate the role of DNA damage in making age and menopausal status as important risk factors in breast cancer.

MATERIALS AND METHODS

Patients

In this study, female breast cancer patients belonging to the age group 30-70, from various hospitals of Tamil Nadu were included. Patients were excluded if any other malignancy was known from their past history. They were divided into 4 groups

Control (Group I)

Consisted of members of the public with no prior history of breast cancer or other cancer related disorders (n=25).

Experimental groups

Group II

Non- metastatic group comprising of breast cancer patients with no evidence of metastasis(n=25).

Group III

Metastatic group consisted of breast cancer patients who at the time of diagnosis revealed evidence of distant metastases (n=25).

Group IV

Post treatment group comprising of patients who had undergone either chemotherapy/ radiotherapy or hormone therapy for their disease (n=25).

Table 1
Clinical details of the study population of breast carcinoma patients

S. No.	Parameter	Numbers
1.	Age range of patients	30-70 yrs
	<50 yrs	32 Nos
	>50 yrs	43 Nos
2.	Age at menarche	12-16 yrs
3.	Age at menopause	46-55 yrs
4.	Menopausal status of patients	
	Pre menopausal	45 Nos
	Post menopausal	30 ,,
5.	Clinical status of patients	
	Non- metastatic breast carcinoma	25 ,,
	Metastatic breast carcinoma	25 ,,
	Post-treatment group	25 ,,

Clinical details of patients were given in Table 1. Informed consent was obtained from every patient.

Sample collection and lymphocyte separation
Blood samples were collected by venous arm puncture from the patients and healthy subjects. 1 ml of heparinized blood was carefully layered over 1 ml of Lympoprep and centrifuged for 35 minutes at 500 xg and 25 ^{0} C. The interface band containing lymphocyte was washed with Phosphate buffered saline (PBS) and then collected by 15 minutes centrifugation at 400 xg. The resulting pellets were resuspended in PBS.

Measurement of lymphocyte DNA damage
DNA damage was assessed in <50 and >50 age groups and also in pre-menopausal and post-menopausal women groups of breast cancer patients following comet assay protocol given by Singh et al [6]. with slight modifications except that electrophoresis was carried out for 25 minutes. After electrophoresis, the slides were stained with 70 µl ethidium bromide / slide, covered with a cover slip and analyzed using a fluorescence microscope. The images of 100 randomly chosen nuclei (50 cells from each of the two replicate slides) were analyzed visually for each subject. The results were expressed as the mean comet tail moment of 50 cells. The comet tail moment is defined as the product of the percentage of cellular DNA in the comet tail and the length of DNA tail migration. The higher the comet tail moment value, the greater the amount of cellular DNA strand breaks.

Statistical analysis
Statistical analysis was performed using Statistical Package for Social Science version 14. Data was presented as mean ± Standard deviation (SD). One-way Analysis of Variance (ANOVA) was used to compare the significance of means, between control and experimental groups. Correlation coefficient was calculated by Pearson's method. P values were considered significant at <0.05.

RESULTS AND DISCUSSION

Lymphocytes were isolated from heparinised blood of breast cancer patients and control subjects using lymphoprep. DNA damage was assessed in <50 and >50 age groups and also pre-menopausal and post-menopausal women groups of breast cancer patients.

DNA damage and age

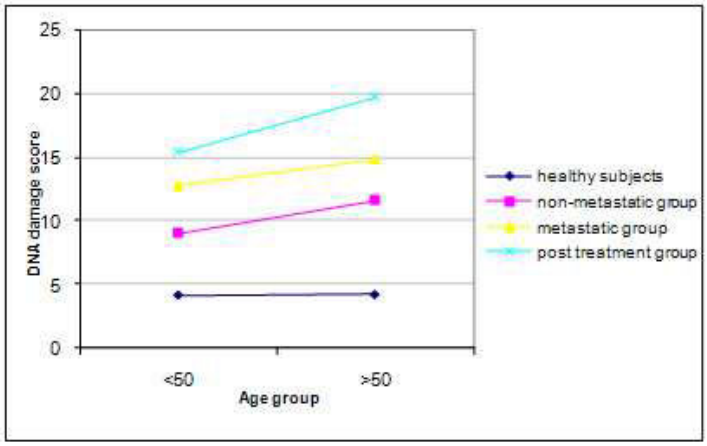

Figure 1
DNA damage scores in healthy subjects and breast cancer patients based on age group

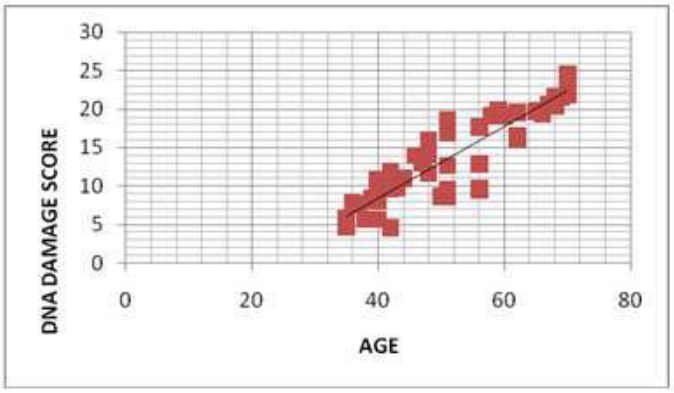

Figure 2
Scatter plot showing a positive association (r= 0.93) between age and DNA damage scores in breast cancer patients

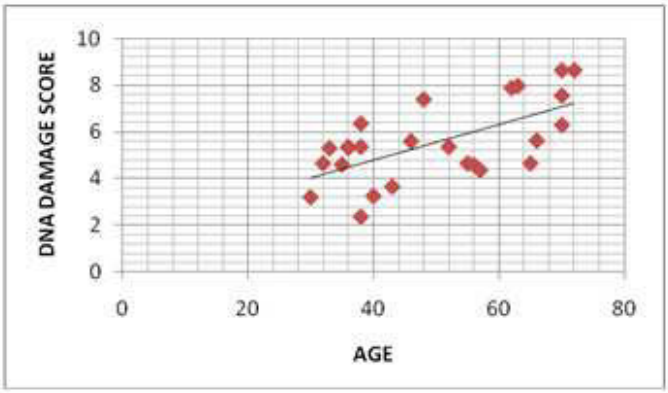

Figure 3
Scatter plot showing a positive association (r= 0.62) between age and DNA damage scores in healthy subjects

DNA damage based on age group is shown in figure 1. In the age group <50 significantly increased DNA damage was found in the non-metastatic (9.03), metastatic (12.81) and post-treatment (15.40) groups respectively when compared with the normal subjects (4.15). Similarly, in the age group >50 significantly increased DNA damage was found in the non-metastatic (11.59), metastatic (14.81) and post-treatment (19.71) groups respectively when compared with the normal subjects (4.19). Thus DNA damage was found to increase progressively from Non-metastatic group (9.03 &11.59) to metastatic groups (12.81 &14.81) and also in post-treatment groups (15.40 &19.71). This clearly indicates that the DNA damage is more pronounced with the progression of disease. When compared between the two age groups of women (<50 and >50), the DNA damage was significantly higher in >50 age group than that of <50 age group in all the stages of disease and control subjects. Correlation studies between age and DNA damage were shown in figure 2 and 3. A positive and significantly higher correlation at p <0.001 level was observed in breasts cancer patients (r= 0.93) than that of control subjects (r= 0.62). Elevated DNA damage in breast cancer patients clearly indicates that the accumulation of DNA damage may contribute to breast carcinogenesis. Higher levels of DNA adducts and oxidative base lesions have been reported in normal adjacent and tumor tissues of breast cancer patients compared with controls[7]. Since elevated DNA damage was found in post-treatment group, it is also evident that to some extent, cancer treatment and therapies also induce DNA damage. High levels of DNA damage are maintained along successive clinical interventions, due to continued production of free radicals during breast cancer treatment, despite the activation of the repair mechanisms that are not sufficient to overcome the effects of the oxidative stress at this level [8]. Hence, this could possibly be a mechanism by which the cancer cells are eliminated during treatment. Furthermore, as shown in Figure 2 a positive association (r = 0.93) has been found between age and DNA damage in breast cancer patients. Aging seems to play a definite role by increasing DNA damage in breast cancer. In the present study a positive correlation (0.62) was also observed in case of healthy subjects but significantly less than the cancer patients. Reports revealed that aging has been associated with damage accumulation in the genome and hence this also contributes to the enhanced DNA damage found in breast cancer patients [9]. A similar study has shown that 80% of breast cancer occurs in women over 50 years [10]. Incidence of breast cancer increased in all ages but more in women older than 50 years [11]. Age contributes to advancement of the disease activity as well. This is evident from the fact that, in metastatic group (group III), increased damage was found in >50 group than that of <50. One contributing factor of aging is increased production of free radicals. High concentration of free radical may contribute to metastasis.

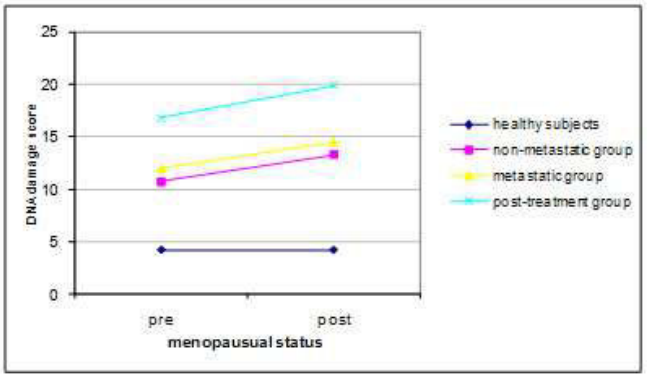

Figure 4

DNA damage scores in healthy subjects and breast cancer patients based on the menopausal status

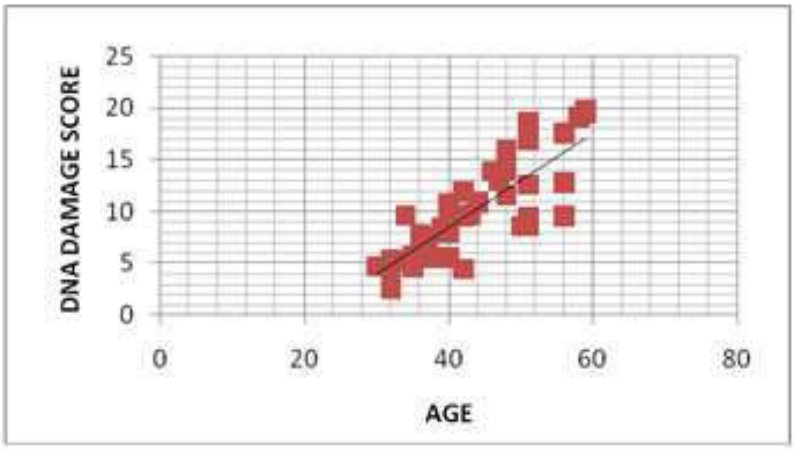

Figure 5

Scatter plot showing the correlation between age and DNA damage scores in pre-menopausal group (r=0.79)

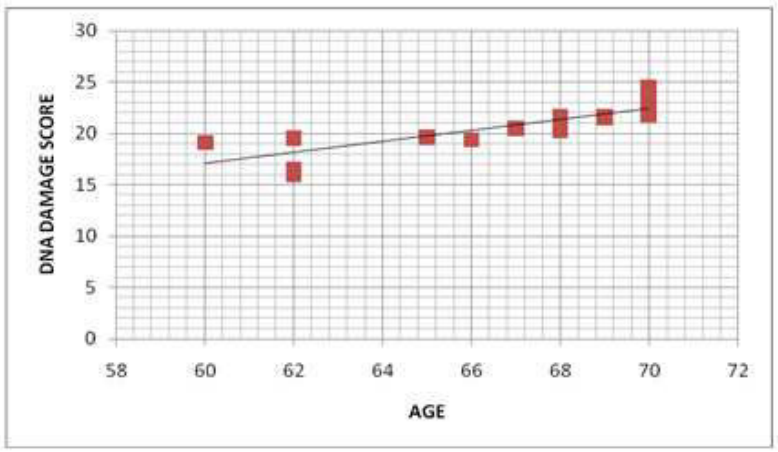

Figure 6

Scatter plot showing the correlation between age and DNA damage scores in post-menopausal group (r=0.85)

Figure 4 shows the DNA damage in two groups based on the menopausal status namely, pre-menopausal and post-menopausal groups. In both the pre and post-menopausal groups, significant increase in DNA damage was found progressively in non-metastatic (10.71 and 10.79), metastatic (14.19 and 14.16) and post-treatment (16.82 and 16.87) groups respectively when compared with the normal subjects (4.17 and 4.16). Furthermore significant increase in DNA damage was recorded in post-menopausal group when compared to pre-menopausal group. As shown in Figures 5&6, higher correlation was found between DNA damage and age in post-menopausal group (r= 0.85) than that of premenopausal group (r= 0.79). Increased DNA damage and the strong positive association with age in post menopausal group may be due to the use of menopausal hormones, a combination of estrogen and progestin, referred to as hormone replacement therapy (HRT)[12]. Longer lifetime exposure to the hormones estrogen and progesterone increases the risk of breast cancer. Mammalian cells convert estrogen into related compounds that not only generate free radicals capable of damaging DNA, but also bind to DNA and pull out a nucleotide base- a process known as depurination. The resulting mutations can convert an innocent cell into a cancerous tumor[13]. Age might also, to some extent play a role in increased DNA damage, as most of the post-menopausal women are over 50 years.

CONCLUSION

DNA damage was in found to be significant in breast cancer patients and seems to increase with the progression of disease to metastasis. Strong positive association have been found in general, between DNA damage and age; which is also more pronounced in post-menopausal group than premenopausal patients. Further investigation will help in understanding the type of DNA damage and the deficient repair systems that prevail in a cancer cell.

CONFLICT OF INTEREST

Conflict of interest- nil

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