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RESEARCH ARTICLE

Assessmento of DNA Damage in Benign and Malignant Breast Tumours

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Summary

Breast is the second most common site of cancer formation. It has been found that in patients with breast cancer DNA instability was enhanced and together with this enhanced damage, the DNA repair capacity which was estimated to contribute to the neoplastic transformation in the breast was decreased. The aim of the present study was to investigate the possible DNA damage in the peripheral lymphocytes among the patients with benign and malignant breast disorders in comparison to healthy controls by using the single cell gel electrophoresis (comet) assay. In this study, DNA damage of 28 patients with benign and 23 patients with malignant breast disorders operated in Gazi University, Medical Faculty, Department of Surgery were compared with 30 healthy controls comparable in sex, socioeconomic status and smoking habits with no history of any breast disease. Significant increases were observed in DNA damage in patients with benign and malignant breast disorders compared to the healthy controls. No significant difference was found between benign and malignant breast disorders.

Key Words: *Breast cancer, Benign breast disease, Single cell gel electrophoresis assay, DNA damage, Comet assay.*

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Beniğn ve maliğn meme tümörlü hastalarda DNA hasarının değerlendirilmesi

Özet

Meme, en sık kanser gelişen ikinci bölgedir. Meme kanseri hastalarında DNAda değişikliklerin arttığı ve bu hasar artışıyla birlikte memede neoplastik dönüşüme katkıda bulunan DNA onarım kapasitesinin de azaldığı bildirilmektedir. Bu çalışmanın amacı, beniğn ve maliğn meme rahatsızlığı bulunan hastaların sağlıklı kontrollerle karşılaştırmalı olarak olası DNA hasarlarını, tek hücre jel elektroforez (comet) yöntemi ile araştırmaktır. Bu çalışmada, Gazi Üniversitesi, Tıp Fakültesi, Cerrahi Anabilim Dalı'nda operasyon uygulanan 28 beniğn, 23 maliğn meme hastası ile cinsiyet, sosyoekonomik sınıf ve sigara içme alışkanlığı açısından eşleştirilmiş meme hastalık hikayesi bulunmayan 30 sağlıklı kontrolün DNA hasarı karşılaştırılmıştır. Beniğn ve maliğn meme hastalarında sağlıklı kontrollere kıyasla DNA hasarının arttığı gözlenmiştir. Beniğn ve maliğn meme hastaları arasında önemli fark bulunmamıştır.

Anahtar Kelimeler: *Meme kanseri, Beniğn meme hastalığı, Tek hücre jel elektroforez yöntemi, DNA hasarı, Comet yöntemi.*

INTRODUCTION

Breast cancer currently accounts for 20% of all female cancers worldwide and is the most frequent malignancy occurring in women (1). Breast cancer etiology encompasses multiple, diverse risk factors, such as environmental agents, hormonal exposures and genetics. Hereditary breast cancer constitutes only approximately 5% to 10% of all breast cancer cases. Overall, more than 90% of breast cancers are sporadic (2,3). Three well-established risk factors, later age at first birth and nulliparity, higher income and family history, account for 40% of breast cancer cases. Therefore, 60% of cases are without defined risk factors (4). Breast carcinomas arise

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from mammary epithelium through a sequence of histological changes from normal epithelium through hyperplasia, atypical hyperplasia, *in situ* carcinoma and finally leading to invasive malignant disease. Several changes at the cellular level usually take place sequentially together with the accumulating genetic alterations leading to changes in the stages of differentiation (5,6). Instability of the genome in human breast cells is an important contributor to heritable and somatic genetic changes that drive to tumorigenic processes (7). Breast carcinomas develop together with a wide range of genetic alterations, and even subtle genetic differences can have a striking effect on clinical outcome. Genetic abnormalities potentially important to tumorgenesis might accumulate in morphologically normal tissues before any histological abnormalities are detectable (8). Genetic alterations have also been noted in other pathologically benign lesions, including various components of fibrocystic change. Genomic instability can proceed to cancer after a period of time, which amplifies even the smallest genetic changes occurring in benign or histologically normal tissue (9-11).

Elevated DNA damage levels or unrepaired DNA damage and suboptimal DNA repair may cause mutations or chromosomal aberrations that contribute to malignant transformation and breast cancer risk. At the cellular and molecular levels, genes whose products participate in complex responses to DNA damage are commonly deregulated or inactivated in tumours (12,13). Such defects in DNA damage recognition, signaling and/or downstream responses, including cell-cycle checkpoints, DNA repair or cell death, allow the genetically unstable cancer cells to survive, proliferate and acquire even more genetic instability (12-16).

Methodologies for measuring DNA damage differ between laboratories and depend upon the DNA-damaging agent used, DNA repair kinetics, the endpoint measured and ways to measure the endpoint. Various biomarkers have been used to determine cellular DNA damage. The single-cell gel electrophoresis, or Comet assay, is a novel approach for the assessment of DNA strand breakage in a single cell. It is based on the alkaline lysis of labile DNA at sites of damage. The assay is relatively easy to perform and well-suited for population-based studies (17-21).

The studies describing the levels of DNA damage in malignant and benign breast tumours are lacking. For this reason; the aim of this study was to investigate the possible DNA damage in the peripheral blood lymphocytes among the patients with benign and malignant breast tumours in comparison with the healthy controls by using the comet assay using a computer based image analysis system.

MATERIALS AND METHODS

Subjects

The study group consisted of 51 patients with benign or malign breast tumours operated between January 2006 and December 2006 in Gazi University Faculty of Medicine Department of Surgery; Ankara, Turkey. The patients were divided into three: first group consisted of patients with benign breast tumours (fibroadenoma and fibrocystic disease), the second group consisted of patients with breast carcinoma and the third group consisted of healthy controls comparable in sex, socio-economic life style and smoking habits without history of breast or other cancers. The preoperative diagnosis of all breast cancer patients were confirmed by tru-cut or ultrasound guided biopsy. The patients; with controversial preoperative diagnosis either with tru-cut biopsy or operative measures, with a history of chemotherapy or radiotherapy or breast or other diseases other than cancer and who have breast disorders in their families were excluded from the study. All the subjects were provided with a written informed consent before the blood samples were drawn from them. The ethical standards provided by the Helsinki declaration were followed and was approved by the local ethical committee. Subjects in all three groups were compared in terms of demographic characteristics, smoking habits and DNA damage in peripheral lymphocytes determined by comet assay.

Blood samples and cell preparation

2 ml heparinized whole blood was collected by venipuncture from patients and controls. Lymphocytes were isolated immediately by Ficoll-Hypaque density gradient (22) and washed with PBS. An aliquot of cells was used to check for viability by trypan blue exclusion.

Comet assay

The basic alkaline technique of Singh et al. (1988), as further described by Collins et al. (1997) was followed (23, 24). For visualization of DNA damage, slides were examined at a 1000X magnification using a 40X objective on a fluorescence microscope. Measurements were made by a computer-based image analysis system 'Comet Assay III' Perceptive Instruments (Suffolk, England). Images of 100 randomly selected lymphocytes were analyzed from each sample and tail length, tail intensity and tail moment were measured.

Statistical Analysis

For statistical analysis SPSS for Windows 10.0 computer program was used. Results were expressed as mean±SE and the statistical comparison of the results from the healthy controls and the patients with benign and malignant breast tumours were performed using one-way analysis of variance (ANOVA) test and post hoc analysis of group differences was performed by the LSD test.

RESULTS

The first group consisted of 28 females with benign breast tumours with a mean age of 37.6 years (23-60 years). 21 patients of this group had fibroadenoma whereas the remaining 7 patient had fibrocystic disease. The smoker and non-smoker ratio was 10/18 in this group. The second group consisted of 23 female patients with breast cancer with a mean age 50.23 years (38-63 years) and smoker/non-smoker ratio was 8/15.

Only excisional biopsy was performed to the patients with benign breast tumours. In the patients with breast carcinomas, according to TNM classification (25); there were 7 patients with stage I, 14 patients with stage II and 2 patients with stage III. In this group, breast conserving operation was performed in 11 and modified radical mastectomy was performed in 12 patients. The third group consisted of 30 healthy females with a mean age 27.4 years (22-46 years) and their smoker/non-smoker ratio was 7/13.

The DNA damage expressed as tail length, tail intensity and tail moment in the lymphocytes of the patients with benign breast tumours and breast cancer and also healthy controls are given in Figure 1. The DNA damage was significantly higher in the patients with breast cancer and benign breast tumours compared to the healthy controls. Although the tail length and tail intensity were found to be higher in the peripheral lymphocytes of the patients with benign breast tumours in comparison to breast cancer patients, there were no statistically significant differences in terms of tail length, tail intensity and tail moment between the patients with breast cancer and benign breast tumours.

DISCUSSION

Breast cancer is an important contributor to morbidity and mortality in the society, but factors that affect the cause of the disease are poorly defined. Breast tissues that have normal histology often have genetic changes that might be important in understanding the local breast environment within which cancer develops. Complex interactions between genetic and environmental factors affect the site of tumor formation and play an important part in early tumorigenesis (8). Increased genetic instability, either spontaneous or mutagen-induced, has been considered as a predisposing factor for neoplastic transformation (26). It has been argued that any situation that increases the mutation rate can also accelerate carcinogenesis (27). In breast cancer as in other human malignancies, mutations affecting multiple genes must occur in order for a normal epithelial cell to acquire the abnormal behaviors that characterize the neoplastic state (28,29). Molecular findings for many types of cancer suggest that genetically altered cells develop from single stem cells that have acquired specific genetic changes and thus have escaped normal growth control (30-32). Genetic alteration accumulates over time and eventually malignant clones evolve through

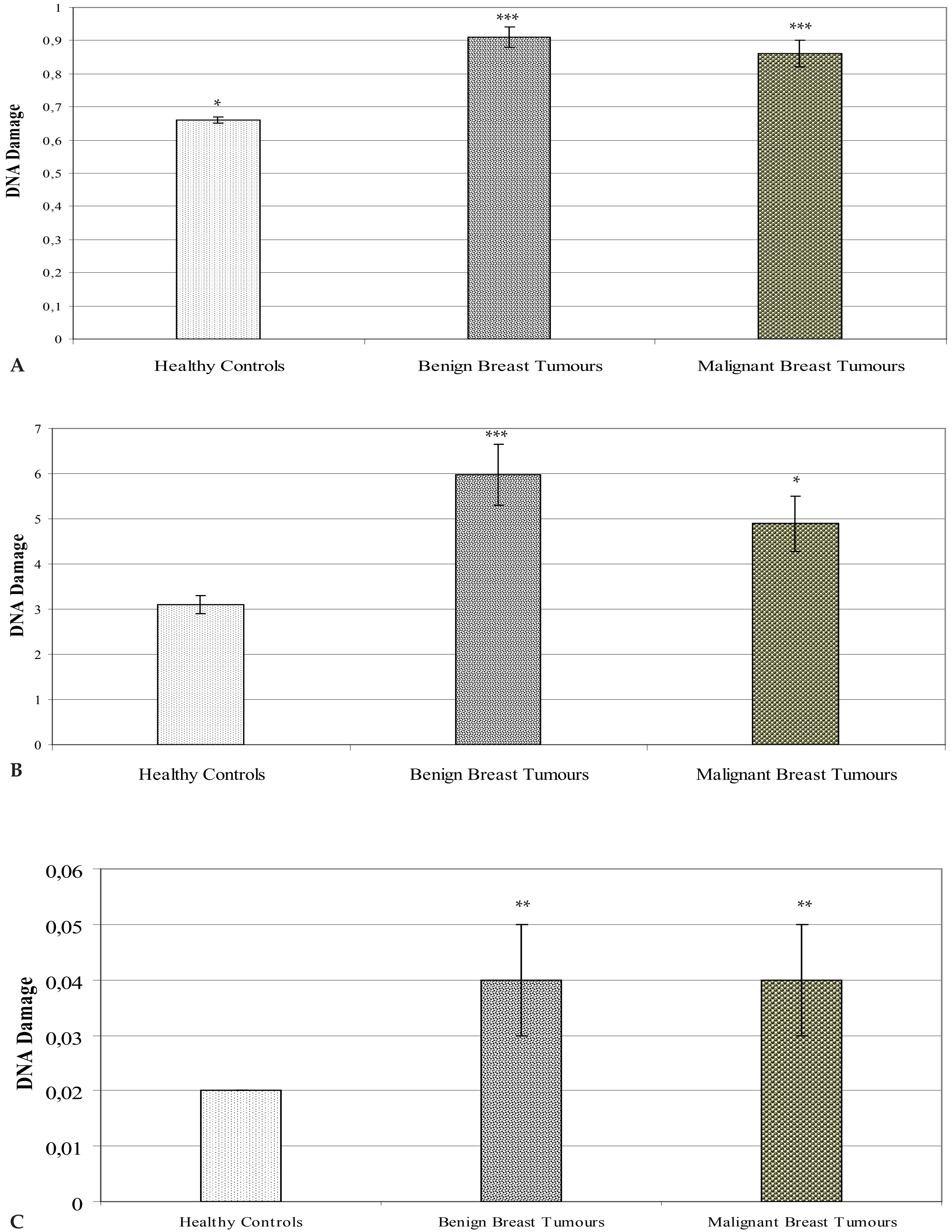

Figure 1. DNA damage in peripheral lymphocytes of the groups expressed as (A) tail length, (B) tail intensity and (C) tail moment (*** p<0.001, ** p<0.01, *p<0.05). The results are given as mean±SE.

histological progression and proliferate in response to endogenous or exogenous factors (33). Early detection of breast cancer reduces the suffering and cost associated with the disease. A sensitive assay to identify markers that can accurately diagnose the onset of breast cancer using non-invasively collected blood samples is ideal for early detection. The earlier and more accurate the diagnostic marker can predict the disease onset, the more valuable it becomes.

In the present study the DNA damage in the peripheral lymphocytes of the patients with benign breast tumours and breast carcinomas were compared with healthy control subjects by comet assay. It was found that the DNA damage in the breast cancer patients and in the benign breast tumours were higher than the healthy controls. Our findings support the hypothesis that DNA damage may serve as a breast cancer risk biomarker. The comet assay used in the present study is sensitive in detecting significant differences in single strand breaks in benign breast tumours and carcinomas. Although there is considerable evidence that individual variation in the detection, signaling, toleration, and repair of DNA damage contributes to human cancer risk, several studies have reported increased thyroid, breast and bladder cancer risks associated with higher DNA damage measured by the comet assay (34-37). Limited previous studies have also shown that breast cancer patients and healthy women with a positive family history may have an increased DNA damage and also DNA repair (38,39). DNA damage checkpoint is activated in a wide variety of human preneoplastic and neoplastic lesions. Increased normal cell proliferation, which results from the response of the preneoplastic and neoplastic lesions, could simply be a reflection of their high proliferation index. Enhanced proliferative index and DNA damage has been shown in normal colonic mucosa by Gorgoulis et al 2005 (40). Although it would be ideal to use target tissue in evaluating DNA damage, peripheral lymphocytes were used in our study as Smith et al 2003; since it is easy and rapid to work with (36).

In the present study, although the difference is not statistically significant, the DNA damage seems to be higher in the patients with benign breast tumours compared to the patients with breast cancer. In the process of DNA repair, the number of DNA strand breaks have been reported to increase. The high DNA damage observed in the benign breast cancer patients compared to malign breast cancer patients can probably be attributed to high DNA repair capacity of these patients. DNA repair capacity is essential for cell survival and the maintenance of cell cycle control. Inter-individual variation in DNA repair capacity has been observed in several in vitro lymphocyte assays (41,42). The difference between DNA damage and repair capacity led to genetic differences leading to susceptibility to carcinogenesis has been shown in many studies. Popanda et al 2003 reported that deficiency in DNA repair is a risk factor for the development of breast cancer; Wu et al 2003 reported that the DNA repair capacity of lung cancer patients was significantly lower than that of controls; Udumudi et al 1998 found that low DNA repair capacity is a susceptibility factor for cervical carcinoma (43-45). Recent studies have shown the DNA damage acts as an anti-cancer barrier. DNA damage response may serve as an inducible barrier to constrain tumor development in its early, pre-malignant stages and create environment that, over time, selects for mutations in checkpoint genes. DNA damage response may rescue defective cell growth, avoid senescence and limit cell death at the expense of genomic instability and tumor progression (40,46,47). Thus, activation of the DNA damage checkpoint occurs specifically in preneoplastic and neoplastic lesions. Some benign breast lesions confer increased risk for carcinoma; therefore, genomic instability in benign breast epithelium might indicate the existence of adverse pathological processes associated with early carcinogenesis. Some benign proliferative lesions, both with and without atypia, seem to contain precursor mutations that might affect neoplastic potential (48,49). Understanding the functional importance of genomic instability in early carcinogenesis has important implications for improvement of diagnostic and treatment strategies. This concept has been formulated, based on the results obtained from analyses of human solid tumours derived from somatic cells, including the various stages of lung, urinary bladder, colorectal,

breast cancer and melanomas, as well as some mouse tumor models and cultured human somatic cells (17,46-48,50).

CONCLUSION

Our results support the hypothesis that increased breast cancer risk is associated with higher DNA damage and evaluation of DNA damage response may contribute to early detection and prevention of breast cancer but due to enhanced proliferative index in certain benign disorders, use of DNA damage only may not be sufficient to show risk of progression to cancer. Studies with larger population and also in other cells apart from lymphocytes are necessary. On the other hand; our results also show that comet assay seems to be reliable, rapid, and sensitive method for detecting DNA damage in individual cells and fulfils the requirements of a biological marker to detect/ prevent breast cancer risk.

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