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Sister chromatid exchange in peripheral blood lymphocytes as a possible breast cancer risk biomarker: A study of Iranian patients with breast cancer

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ABSTRACT

Introduction: Sister chromatid exchanges (SCEs) can be induced by various genotoxic treatments, suggesting that SCEs reflect a DNA repair process and it may be a good index for assessment of genomic instability. However, the occurrence of genetic instability and in particular, of spontaneous SCEs has been strongly linked to cancer. Several chromosomal regions and many genes have been implicated in breast cancer.

Materials and Methods: Blood samples were obtained from 31 Iranian breast cancer patients and 11 healthy women. SCE was measured in peripheral blood lymphocytes by adding to Ham'sF10 medium in presence of PHA, BrdU (5-bromo-deoxy Uridine) fluorochrome Hoechst 33258, exposure to UV light and Giemsa staining. Then, SCE frequencies of patient and control groups were compared by the Mann-Withney U-test.

Results: Significantly difference was observed between two groups ($p<0.001$).

Conclusion: This study indicates that SCE can be used as a risk biomarker for breast cancer.

Key Words:

SCE, breast cancer, peripheral blood, BrdU.

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INTRODUCTION

Breast cancer is the most prevalent malignancy in women, with about one million cases diagnosed annually worldwide. Many environmental and biological factors have involved in progression of this cancer. Thus, because of genetic heterogeneity, molecular genetic testing is very difficult to identify its causes. For example, various types of molecular methods have been used for detection of BRCA gene

mutations and more recently, CGH and multicolor fluorescence in situ hybridization (M-FISH) have been applied to identify many gains and losses of DNA sequences and loci in breast tumors.[1]

The phenomenon of sister chromatid exchange (SCE) is a symmetrical exchange of apparently identical portions of chromosomes and involves DNA breakage and reunion mechanism[2]. Cytological assessment of SCE levels in peripheral blood lymphocytes is used as an index of the mutagenic potential of environmental factors such as various mutagenic and/or carcinogenic chemicals like mitomycin C[3-8] and possible therapeutic agents like resveratrol[9] and this test is widely used as a reliable and sensitive indicator of chromosome (DNA) instability, since the SCE patterns can reveal a general genomic instability[8,10]. The increased frequency of spontaneous SCE has been reported in patient with various neoplastic diseases[10,11]. Variations in DNA repair mechanisms or detoxifying associated with cancer[12]. Also, the sister chromatid exchange frequency was found to be significantly higher in individuals with Werner syndrome, Bloom's syndrome and myelodysplastic disease than in their control groups. These diseases are known to be associated with genomic instability[13]. More importantly, 10 SCEs occur spontaneously in normally cycling human cells.[14,15]

Whereas tumor markers have been widely investigated, there have been few studies on the predictive value of cytogenetic biomarkers for cancer development. The aim of this study was to assessment of SCE frequency in Iranian breast cancer patients.

MATERIALS AND METHODS

Patient and Control Samples:
This research was done in Tehran University of Medical Sciences, Iran. Thirty one affected females with breast cancer between 26 and 65 years of age were examined as the study group. On the other hand, 11 healthy women as the control group were studied. Heparinized blood was obtained by venipuncture of an arm from samples and informed consent was obtained from all participants. They did not smoke and were not taking drugs for medical or other reasons.

Slide Preparation and SCE Test:
Whole blood cultures were initiated by adding 10 drops of blood to each culture flask containing 5 mL of Ham'sF10 supplemented with 15% fetal calf serum, 4 mM L-glutamine, 100 units/mL penicillin, 100 g/mL streptomycinand0.1 mL of phytohemagglutinin (PHA) (Biochrom) for 72 hrs at 37 ºC. 5-Bromodeoxyuridine (BrdU) (Sigma, St. Louis, MO, USA) was added at a final concentration of 10 µg/ml for the last 48 hrs of incubation, during which time the culture tubes were kept in the dark to minimize SCE induction by photolysis of BrdU-substituted DNA. Colcemide (0.02 mg/ml) was added for the final 40 min of culture. Harvesting of cells and slide preparation were accomplished by the standard method[16]. The slides were stained for 12 min in a 0.5 µg/ml solution of the fluorochrome Hoechst 33258, exposed to UV light (distance approximately 12 cm) for a minimum of 2 hrs in a sodium phosphate buffer (0.3 M; pH 7.0), rinsed and stained with 4% Giemsa at pH 6.8.

The microscope slides were studied.

The number of SCEs/chromosome was calculated for each individual. SCE frequencies (number per chromosome) were determined in cultures from different groups. Thirty metaphases were studied for each individual; 930 and 330 metaphases were analyzed in 31 patients and 11 healthy individuals, respectively.

Statistical Analysis:

The distribution of the number of SCEs/chromosome was examined for two groups. The mean SCE frequency was evaluated and compared to the control group statistically by the Mann-Withney U-test.

RESULTS

The mean frequencies of spontaneous SCE per metaphase were 6.67 ±1,42 and 4.64 ± 1.19 in patients and control group, respectively. Spontaneous SCE values in the study and control groups were found statistically significant at the end of the evaluation ($p<0.001$) (Table 1).

Table 1: Mean frequencies of SCE per methaphase for two groups.

Groups	Metaphase number/ number of individual	Age limit (year)	SCE/ metaphase (mean±SD)
Patients	930/31	26-65	6.67±1.42
Control	330/11	28-60	4.64±1.19

Table 2 shows the mean SCE frequencies in patients with breast cancer in previous studies and comparison with present study. One metaphase with 8 SCE of patient has been shown in (Figure. 1)

Table 2: Comparison of mean SCE frequencies in previous studies.

Published year	Patients SCE/ metaphase (mean+ SD)	Control groups SCE/ metaphase (mean+ SE)
Sholnick et al.[17]	7.21 ± 0.35	5.1 ± 0.77
Livingston et al.[18]	6.98 ± 0.32	4.7 ± 0.18
Adhvaryu et al.[19]	7.77 ± 0.41	6.28 ± 0.87
Husain et al.[20]	5.96 ± 0.19	4.62 ± 0.14
Dhillon et al.[11]	5.8 ± 0.23	4.65 ± 0.19
Present study	6.67 ± 0.05	4.64 ± 0.07

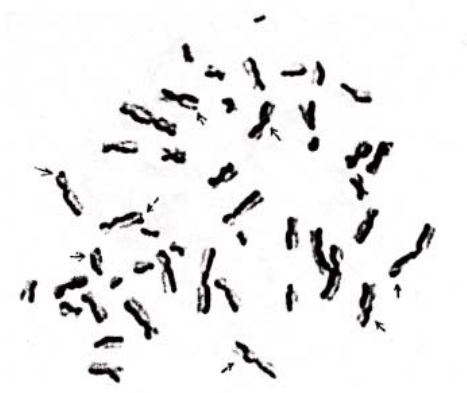

Fig. 1: Sister chromatid exchange.

DISCUSSION

The application of cytogenetic biomarkers (chromosomal aberrations, sister chromatid exchanges and micronuclei) is critical for health risk assessment after environmental or occupational exposure. We studied spontaneous SCE frequencies in the lymphocyte cultures of 31 breast cancer patients and 11 controls in Iranian population. Higher spontaneous SCE were observed in

the cells of patients compared with the controls ($p< 0.001$). Similar elevations of spontaneous lymphocytic SCE rates have been reported by others for patients with malignant lymphoma[21], cutaneous malignant melanoma[22], lung cancer[23] and uterine cervix cancer[24,25] and breast cancer.[10,11]

However, SCE provides an easy, reproducible and good index for monitoring DNA damage and DNA repair status[3,26]. The analysis of sister chromatid exchanges is a sensitive tool for evaluating DNA lesions of the kind that may lead to cancer development and has been well accepted for studying carcinogenic and/or mutagenic potentials of chemical as well as physical agents[6-9,25]. In human neoplasms, spontaneous SCE study has been used for various purposes[27] and it may indicate a promising future for treatment monitoring.

Although genomic instability is a common feature of cancers, its exact mechanism is not understood yet; however, data support the hypothesis that chromosome instability may be related to the development of neoplasia. This hypothesis is supported by the fact that spontaneous SCE frequencies from various cancers have shown higher.[8-10,20,21,28]

Various factors have been suggested as capable of influencing the frequency of SCEs in human lymphocyte cultures. These include possible technical artifacts (e.g., the concentration of BrdU, the number of lymphocytes in the sample and exposure to light following incorporation of the analog) as well as biologically more important considerations such as heavy cigarette smoking, the presence of a malignancy and/or chemotherapeutic treatment and occupational exposure to agents that induce SCEs. Although the molecular mechanisms responsible for the production of SCEs are not still fully understood, recently, it was proposed that a double strand break (DSB) generates a 'signal' which triggers the cell to make a recombinogenic exchange in a looped structure either within (intra-chromatid) or between (inter-chromatid) sister chromatids.[29]

Our results indicates that the increased mean SCE frequency in breast cancer patients compared with the controls ($p< 0.001$), suggesting SCE can be used a preclinical marker for early detection of breast cancer.

In wild-type mammalian cells, the repair of DSB by homologous recombination uses mainly the sister chromatid as a template[30]. BRCA1 and BRCA2 proteins have an important role in the control of homologous recombination and double strand break repair[31]. Previous data support this hypothesis that enhanced sensitivity of peripheral blood lymphocytes from women carrying a BRCA1 mutation towards the mutagenic effects of various cytostatics[11]. Proteins involved in DNA repair pathways such as p53, BRCA1 and BRCA2 are involved in tumor initiation and its progression. Since, SCE is due to defect in repairing of the damaged DNA, the defect can be in any of the repair pathways which are associated with cancer development and because these proteins have important roles in breast cancer[28], it can imply that SCE may cause breast cancer. Further investigations are needed to be carried out on various types of cancers in order to resolve the mechanism involving SCE and genomic instability associated cancers.

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