Cytogenetic Radiosensitivity of $G_0$-Lymphocytes of Breast and Esophageal Cancer Patients as Determined by Micronucleus Assay

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Radiosensitivity/Human lymphocytes/Breast cancer/Esophageal cancer/Micronuclei.

Enhanced chromosomal radiosensitivity is a feature of many cancer predisposition conditions, indicative of the important role of chromosomal alterations in carcinogenesis. In this study the cytokinesis-blocked micronucleous assay was used to compare the radiosensitivity of blood lymphocytes obtained from Iranian breast or esophageal cancer patients ($n = 50$, $n = 16$; respectively) with that of control individuals ($n = 40$). For each sample, one thousand binucleate lymphocytes were analyzed before and after in vitro exposure to 3 Gy of $\gamma$ rays. The radiation-induced frequency of micronucleus was significantly higher in the breast cancer group ($261/1,000$ binucleated cells) than in esophageal cancer group ($241/1,000$ binucleated cells, $P < 0.01$) or in the control group ($240/1,000$ binucleated cells, $P < 0.01$). The results indicate that breast cancer patients are more radiosensitive compared to normal healthy individuals or esophageal cancer patients. Increased radiosensitivity could be due to defects in DNA repair genes involved in breast cancer formation. Since patients with esophageal cancer did not show elevated radiosensitivity, it is assumed that the contribution of radiosensitivity-related genes to the development of esophageal cancer may be smaller than the contribution of those genes to breast cancer.

INTRODUCTION

The genetic integrity of human population is under constant threat due to exposure to different physical and chemical agents, which could result in more cases of cancers than ever. Although our understanding of chromosome structure is yet incomplete, there are evidences suggesting that chromosomal abnormalities are a direct consequence of damage at DNA level. For instance, chromosome breaks may result from unrepaired double strand breaks in DNA.\(^1\) It has also been understood that chromosome loss and non-disjunction are important steps in carcinogenesis and aging which may be due to the defects in the spindle fiber, centromere or as a result of premature condensation of chromosome structure before metaphase.\(^2,3\) Indeed, the elevated sensitivity to the induction of aberrations by carcinogens is a feature of many heritable conditions that cause cancer predisposition.\(^4\) Earlier it seemed that carcinogen sensitivity was different and specific among cancer-prone conditions, for example cells from xeroderma pigmentosum patients are sensitive to UV,\(^5\) ataxia-telangiectasia to ionizing radiation,\(^6,7\) and Fanconi anemia to DNA cross-linking agents.\(^6,8\) Nevertheless, it is now believed that sensitivity to ionizing radiation is detectable not only among patients with the above-mentioned cancer prone syndromes,\(^5–8\) but also among many other cancer prone groups, such as Down’s syndrome,\(^9,10\) Li-Fraumeni syndrome,\(^11\) and Wilm’s tumor.\(^12\) This is probably because ionizing radiation causes a wide range of DNA lesions that overlap with those induced by other specific carcinogens.\(^1,3\) However, by the improvement of new assays even small differences in radiosensitivity can now be detected. Furthermore, there are different mechanisms resulting in enhanced chromosomal radiosensitivity like defects in DNA repair,\(^14\) different chromatin structures,\(^15,16\) or cell cycle checkpoints.\(^17\) Chromosomal radiosensitivity is therefore an important biomarker of cancer predisposition.

Currently there are many assays to determine the chromosome damages, among which cytogenetic techniques are more practical. In the classical cytogenetic technique, chromosomes are studied directly by observing and counting aberrations in metaphases.\(^18\) This method provides the most detailed analysis but due to the complexity and laboriousness of analyzing aberrations, using a simpler system of measuring chromosome damage, e.g., micronucleus (MN)
assay is suggested.\textsuperscript{19} MNs are expressed in dividing cells that either contains acentric fragments and/or whole chromosomes that failed to be incorporated in two daughter nuclei. MNs, therefore provide a convenient and reliable index of both chromosome breakage and chromosome loss.\textsuperscript{19–21}

Since MNs are expressed in cells that have completed nuclear division, they are ideally scored in binucleated cells. Cytochalasin-B treatment enables the accumulation of virtually all dividing cells at the binucleate stage in dividing cell populations regardless of their degree of synchrony and the proportion of dividing cells.\textsuperscript{22,23} It has been shown conclusively that the cytokinesis-blocked micronucleus (CBMN) assay can detect from 60% to 90% of acentric fragments.\textsuperscript{24}

Many research groups have applied this method for assessing radiosensitivity of G\textsubscript{2}-lymphocytes from healthy donors,\textsuperscript{25–27} although some other studies did not find any correlation between lymphocyte radiosensitivity and either acute or late effects of radiotherapy in head and neck cancer patients.\textsuperscript{28} Dunst and Gebhart\textsuperscript{29} observed patients with radiation hypersensitivity in lymphocyte tests \textit{in vitro}. This technique has also been used to monitor populations exposed to industrial chemicals\textsuperscript{30} and chemotherapeutic agents.\textsuperscript{31} These findings support the idea that on the basis of MN test, it is possible to identify a group of more radiosensitive individuals.\textsuperscript{24–26}

The purpose of this study was to evaluate the intrinsic radiosensitivity of lymphocytes from patients suffering from breast or esophageal cancer; the latter is the most common cancer in Northern Iran,\textsuperscript{32} in comparison with healthy donors using the CBMN assay.

**MATERIALS AND METHODS**

Heparinized blood samples (5 ml) were obtained from each individual. The control group comprises of 40 healthy individuals, 13 men and 27 women who ranged in age from 24 to 62 years (38.1 ± 9.4) without prior exposure to antibiotics and radiation for at least three months. They did not have any previous history of smoking and alcohol consumption. The esophageal cancer group comprises 16 patients including 9 men and 7 women who ranged in age from 38 to 71 years (52.2 ± 9.7). The breast cancer group comprises 50 patients, 1 man and 49 women, who ranged in age from 25 to 78 years old (46.9 ± 11.4). All the cancer patients were attending different oncology departments in Tehran and Babolsar cities for the post-operative chemotherapy or radiotherapy; none had any evidence of metastatic disease or had any exposure to cytotoxic chemotherapy or radiotherapy prior to blood donation. They were all non-smokers. This study was approved by the Ethical Committee of Tarbiat Modares University and an informed consent was obtained from each individual before collecting blood.

**Irradiation and cell culture**

Irradiation was performed with \textsuperscript{60}Co \textgamma-rays (Teratron 780, Canada) at a total dose of 3 Gy with a mean dose-rate from 68.8 to 76.4 cGy/min at room temperature (23 ± 2°C).

After irradiation, lymphocytes were cultured at 37°C for 90 hours in RPMI-1640 medium containing 15% FCS, penicillin (100 iu/ml), streptomycin (100 µg/ml), and L-glutamine plus 1.5% PHA (Gibco BRL). At 44 h cytochalasin-B (Sigma) was added to yield a final concentration of 3.5 µg/ml and the cells were incubated for an additional 46 h. The cells were then harvested, fixed with methanol and acetic acid (1:6 the first time and 1:25 the next 2 times). After the last centrifugation, cells were resuspended in a small volume of fixative and dropped onto pre-cleaned slides and stained with 10% Giemsa. The scoring criteria were those indicated by Fenech.\textsuperscript{21} One thousand binucleate lymphocytes were scored at the magnification of × 400 for each sample. Also for each sample a duplicate set of cultures were processed and analyzed.

**Statistical analysis**

For each sample the spontaneous MN yield was subtracted from the yield in irradiated cells to give the induced MN

| Subjects                  | Total No. of subjects | Mean age ± SD (Range, Yrs) | Mean MN frequency/1000 binucleated cells ± SD |
|---------------------------|-----------------------|-----------------------------|-----------------------------------------------|
|                           |                       | Before irradiation          | After 3 Gy irradiation                        | Induced MN frequency |
| Controls                  | 40                    | 38.1 ± 9.4 (24 – 62)        | 18.2 ± 5.8                                   | 258.3 ± 22.9          | 240.4 ± 18.5           |
| Esophageal cancer patients| 16                    | 52.2 ± 9.7 (38 – 71)        | 17.0 ± 3.4*                                  | 258.6 ± 18.2*         | 241.0 ± 18.9*          |
| Breast cancer patients    | 50                    | 46.9 ± 11.4 (25 – 78)       | 23.5 ± 4.4**                                 | 282.3 ± 21.3**        | 261.2 ± 17.1**         |

* Not significantly different from controls (p > 0.05)  
** Significantly different from controls and esophageal cancer patients (p < 0.01)
yield. Using SPSS (version 11.5) statistical software, paired *t*-test was used to compare the frequency of MN yield within a group and between groups before and after irradiation. The Kolmogorov-Smirnov test showed a normal distribution of data. Since the number of subjects studied was not similar in the three groups, the frequencies of CBMN were also compared using non-parametric Mann-Whitney U-test and Kruskall-Wallis tests.

**RESULTS**

The data obtained from normal healthy controls, breast or esophageal cancer patients are summarized in Table 1 and Figs. 1 and 2. The mean spontaneous MN yields for the controls, breast or esophageal cancer cases were found to be about 20/1,000 binucleated cells. Whereas, the frequencies largely increased to about 260–280/1,000 binucleated cells after irradiation with 3 Gy of γ-rays. Among the normal individuals and esophageal cancer patients there was no significant difference in the net increase between men and women (p > 0.05), therefore data for both sexes were combined in the following statistical analysis.

The mean MN yield for breast cancer patients was significantly higher than that for the control or esophageal cancer groups not only in the spontaneous frequencies but also in the radiation-induced ones (Table 1 and Fig. 1). By contrast, the MN yield for esophageal cancer group did not differ with that in the control group in both spontaneous and after radiation exposure (Table 1 and Fig. 2). Although there was a significant difference in the mean age between the control group and breast or esophageal cancer groups, no age effect was observed in the yield of radiation-induced MN in any group (Figs. 1 and 2).

The histograms in Figs. 3–5 show the distribution of individuals with variable number of radiation-induced MN. The mean + 2SD of the induced frequency of MN in the control group was used as an arbitrary cut-off point as suggested by Scott *et al.*[^33^] As shown in figures, 10% of individuals in the control group (Fig. 3), 30% in the breast cancer group (Fig. 4), and 12.5% in the esophageal cancer group (Fig. 5) were regarded as showing elevated radiosensitivity.

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[^33^]: Scott *et al.*[^33^]
DISCUSSION

The MN assay can be used for normal cells to determine the intrinsic radiosensitivity of individuals, also for monitoring base-line chromosome aberration frequency in unexposed population\textsuperscript{34} and those exposed to low-level radiation.\textsuperscript{35} The present data show that elevated spontaneous frequency of MN in the breast cancer group compared with the control group is in line with the previous results obtained by other investigators.\textsuperscript{36–38} Also some 10\% of normal individuals were found to show increased sensitivity to ionizing radiation (Fig. 3), which was previously reported by Scott \textit{et al}.\textsuperscript{39}

Our observation show that the mean frequency of radiation-induced MN was significantly higher (p < 0.01) in breast cancer patients than in normal controls and this sensitivity was not age related (Fig. 1). As shown in figure 4, about 30\% of the breast cancer patients appeared as more sensitive to radiation, which is similar to the results reported by other investigators.\textsuperscript{33,37,39,40}

In contrast, the mean frequencies of both spontaneous and radiation-induced MN in esophageal cancer patients did not differ significantly compared with those in the control group (Fig. 2). We interpret the results as indicating that genetic component sensitive to ionizing radiation for the development of esophageal cancer is less important compared to that of breast cancers. It is understandable that esophageal cancer is more closely related to exposure to exogenous agents present in food (diet).\textsuperscript{41–43} Nevertheless in the case of lung cancer smoking habit is the critical factor. This notion is in line with the observations with G\textsubscript{2}-assay that patients with breast, colorectal, head and neck, and childhood cancers\textsuperscript{44} as well as patients with brain tumors\textsuperscript{45} exhibited degrees of enhanced sensitivity to chromosome damaging effects of ionizing radiation. While other cases such as cervical or lung cancer did not show elevated radiosensitivity\textsuperscript{44} in which viral infection or smoking, respectively, is believed to be the major etiologic factors.

Present results indicate that mutations in some genes involved in DNA repair may be associated with development of breast cancer. It seems likely that genetic and / or epigenetic alterations of genes are frequently involved in the formation of sporadic, non-hereditary breast cancers. Because, G\textsubscript{0}-lymphocytes of breast cancer patients showed elevated radiosensitivity compared to the cells from normal healthy individuals or esophageal cancer patients, MN assay may have the potential for predicting predisposed cases for breast cancer. By contrast, the assay does not seem helpful for screening cases predisposed for esophageal cancer.

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