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Fibroadenoma and breast cancer patients' lymphocytes sensitivity to gamma rays- An evaluation by micronuclei assay

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ABSTRACT

Micronuclei [MN] in lymphocytes of patients suffering from fibroadenoma [n=29] and breast cancer [n=37] was compared with the data from healthy individuals [n=10]. Healthy controls lymphocytes were exposed at G_0 stage to different doses [0.5 Gy, 1.0 Gy, 2.0 Gy and 4.0 Gy] of γ-rays. Lymphocytes were cultured for 72 hours to obtain MN with cytochalasin B. A dose dependent increase in the incidence of MN frequency and a decrease in the survival of lymphocytes. Frequency of PBLs [Peripheral blood lymphocytes] with one, two, three and four MN is analyzed. In comparison to baseline, the radiation exposed samples showed higher frequency of MN in control, fibroadenoma and breast cancer. The degree of increase was 4.7 times in control, 10.8 in fibroadenoma and 15.2 in breast cancer as compared to samples without radiation. The overall degree of increase in MN frequency from control to breast cancer was 3.2 times. Non irradiated and irradiated samples showed an increase in MN frequency from stage I to stage IV. Influence of age on MN frequency was not significant. MN assay is an additional diagnostic, prognostic tool in benign and malignant tumors of breast to assess the degree of genetic damage and radiosensitivity.

Key words: Breast Cancer, Cytochalasin B, Fibroadenoma, Lymphocyte Culture, Micronuclei.

INTRODUCTION

Genetic risk related to environmental exposure to DNA damaging agents like diet, radiation, therapies, tends the researchers to develop reliable tests to assess acceptable level of genotoxicity and sensitivity of an individual. Peripheral blood lymphocytes [PBL] are the most radiosensitive mammalian cells, they show only a limited repair of radiation induced DNA damage. Cultured human lymphocytes provide a good in-vitro model for studying dose-related induction of genetic changes in the DNA after exposure to ionizing radiation. The frequency of micronuclei [MN] in peripheral blood lymphocytes [PBL] is extensively used as a biomarker of chromosomal damage and genome stability in human populations. Much theoretical evidence has been accumulated supporting the causal role of MN induction in cancer development, although prospective cohort studies are needed to validate MN as a cancer risk biomarker. MN assay has also been applied to identify the dietary and genetic factors that have a significant impact on genome stability [1]. The presence of an association between MN induction and cancer development is supported by a number of observations like, high frequency of MN in untreated cancer patients and subjects affected by cancer- prone congenital diseases, eg, Blooms syndrome or ataxia telangiectasia [2, 3]. Evaluation of the spontaneous genetic damages in circulated lymphocytes of newly diagnosed cancer patients by using cytokinesis-block micronucleus [CBMN] assay, with respect to the factors that might affect micronucleus frequency [i.e. age,

gender, smoking habits and cancer sites] showed significant increase in MN frequency, may be associated with an increase of chromosomal instability in peripheral blood lymphocytes, irrespective of gender, cigarette smoking and cancer sites [4].

The results of this study provide preliminary evidence that MN frequency in PBL is a predictive biomarker of cancer risk within population of healthy subjects. The current wide-spread use of the MN assay provides a valuable opportunity to apply this assay in the planning and validation of cancer surveillance and prevention programs [5]. MN frequency in oral cancer as a surrogate biomarker of cancer in chemoprevention trials [6], correlation between genotoxic MN inducing agents and carcinogenicity e.g, ionizing and ultraviolet radiation [7], MN frequency with the pregnancy complications, cancer and cardiovascular diseases [8, 9] and also associations with other diseases such as Alzheimer's disease, Parkinson's disease and diabetes have been reported [10, 11]. Inverse correlation between MN frequency and the blood concentration and/or dietary intake of certain micronutrients associated with reduced cancer risk such as folate, calcium, vitamin E and nicotinic acid has been reported [12]. Micronucleus scoring was carried out in benign [fibroadenoma] malignant [infiltrating ductal carcinoma] breast lesions FNAC smears to evaluate the role of MN as a biomarker. [13]. Significant reduction in MN frequency was observed with the protective effect of Zingerone a dietary compound against radiation induced genetic damage and apoptosis in human lymphocytes [14].

Several studies have shown that after exposure to ionizing radiation and clastogenic chemicals, the DNA content and the size of most micronuclei showed strong correlation with the frequency of acentric fragments [15]. Currently information is rather limited on the molecular process involved in the production of micronuclei and the quantitative relationships between the frequency of chromosome aberrations and the subsequent micronuclei formation. Studies have shown that acute and late tissue reactions in patients treated with identical dose of radiation vary considerably which could arise from inter- individual variation in radiosensitivity.

MATERIALS AND METHODS

Five milli liter of heparinized venous blood was collected under sterile conditions from healthy individuals [n=10], patients with fibroadenoma [n=29] and patients suffering from breast cancer [n=37] and the patients included in the study were not undergone for any treatment prior to the sample collection. The sample size in this study are small because this is a preliminary study and stage dependent comparison will be carried out with large number of samples in different age groups. To each of the culture vials 0.5 ml of blood was put and irradiated with γ -rays at different doses [each with 0.5 Gy, 1.0 Gy, 2.0 Gy or 4.0 Gy] within one hour of collection blood. Irradiation was carried out employing telecobalt unit Theratron 780C, AECL, Canada, available at the Department of Radiotherapy of Kasturba Hospital, Manipal, with mean dose-rate of 261.82 cGy/min at 78.5 cm SSD [average energy of gamma rays 1.25 MEV].

Cultures were set after the addition of phyto haemagglutinin, and maintained at 37°C for 48 h [16]. After 48 hours of incubation Cytochalasin B was added to the culture vials at a final concentration of 4µgm/ml. Cultures were further incubated for 24 hours. At 72 hours of incubation cells were centrifuged at 800 RPM for 10 minutes and 10 ml of pre warmed 0.56% KCl was added to the pellet and incubated for 5 minutes. Then the cells were fixed with 3:1 methanol: acetic acid [v/v]. Fixative was changed thrice to remove RBCs and to dehydrate the cells completely.

Lymphocytes were obtained from fixed cell suspensions dropped on clean chilled slide, each of which was air dried. Slides were stained with Giemsa solution [6%] for 10 min and analyzed to score the frequency of micronuclei in binucleate cells as per published guidelines [17]. Slides were coded and scored by single observer. One thousand binucleate lymphocytes were scored per subject.

The data from healthy control set of samples helped in the selection of dose. Incidence of micronuclei showed a dose-dependent increase with an increase in the radiation dose from 0.5 Gy to 4.0 Gy. There was an inverse relationship between radiation dose and the viability of irradiated cells, as observed from preparations using trypan blue. Taking cell viability and frequency of micronuclei it was proposed to expose cells to γ-rays at a dose of 2 Gy for further experimentation. Data obtained from healthy set of controls, non irradiated and irradiated [0.5, 1, 2, 4 Gy] were subjected to regression analysis in order to obtain the dose response relationship.

Linear regression was calculated as C + α D, and linear quadratic function was calculated by the formula C + αD + βD^2; where C, α and ß denoted estimated parameters for spontaneous and radiation-induced micronuclei, and D, the radiation dose in Gy. To identify the degree of radiosensitivity the rate of increase of MN frequency from baseline to radiation induced levels was recorded. Degree of increase in micronuclei frequency was categorized into ≤ 5, 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, 36-40, 41-45 times, by introducing a step of "5 times" at each level. Based on this it was found that 60% of control subjects belonged to the ≤ 5 times level , the micronuclei frequency from baseline to radiation induced level increased by 5 times or less. Since majority of the controls belonged to this range of "minimum radio sensitivity", arbitrarily we proposed this as the cut off level. Further in order to streamline the analysis of data, ≤5 times of increase in MN frequency was considered as radioresistance, 6-15 times of increase in MN frequency was considered as "moderately radiosensitive" , and >15 times was considered as highly radiosensitive.

All the tests were carried out using SPSS 4.0 statistical software. Mean, standard deviation, standard error and 95% confidence interval of data were calculated using descriptive statistics. One-way ANOVA test was applied to measure the distribution of MN in different subgroups. Influence of age of patients on MN frequency was assessed using multivariant ANOVA test.

RESULTS

Optimum number of MN with good cell survival rate was observed at the final concentration of 4 µgm/ml cytochalasin B which was used throughout the study.

A dose-dependent decrease in survival of cells was observed with increase in the radiation dose. Cell viability tested at 72 hours of culture using trypan blue was 75% with 0.5 Gy, 70% with 1.0 Gy, 64% with 2.0 Gy and 40% with 4.0 Gy of γ-rays. The incidence of MN was found to increase with increase in radiation dose which was fitted to linear model. Per thousand binucleate lymphocytes, 14, 18, 20 and 34, MN on exposure to 0.5Gy, 1.0 Gy, 2.0 Gy and 4.0 Gy of γ-rays respectively. A dose of 2.0 Gy of radiation was chosen for further experimentation, taking cell viability and MN into consideration.

Table 1: Binucleated peripheral blood lymphocytes with Micronuclei /1000 Binucleated PBLs scored per subjects on exposure to γ-rays [2 Gy]

Group	Sample size [n]	Non-irradiated mean± SD	Irradiated mean± SD
1. Control	10	5.00 ± 2.16	22.88 ± 5.79
2. Fibroadenoma	29	5.64 ± 2.21	58.94 ± 21.08
3. Breast cancer [Pooled from all the stages]	37	11.85 ± 7.39	177.5 ± 105.7
Stages of Breast cancer			
4. Stage I	07	5.63 ± 1.69	94.8 ± 40.55
5. Stage II	11	10.50 ± 8.91	113.4 ± 48.9
6. Stage III	09	11.11 ± 3.40	177.2 ± 67.72
7. Stage IV	10	17.92 ± 6.19	301.1 ± 87.36

Non-irradiated: P<0.05: [3 vs 1,2] [7 vs 1,4,5]
Irradiated: P<0.05: [3 vs 1,2] [7 vs 1,4,5,6]

Table 2: Binucleated peripheral blood lymphocytes with one Micronuclei /1000 Binucleated PBLs scored per subjects on exposure to γ-rays [2 Gy]

Group	Sample size [n]	Non-irradiated mean± SD	Irradiated mean± SD
1. Control	10	4.40 ± 2.11	19.30 ± 4.50
2.Fibroadenoma	29	5.26 ± 1.90	52.26 ± 19.95
3. Breast cancer [Pooled from all the stages]	37	10.41 ± 5.96	158.00 ± 98.2
Breast cancer			
4. Stage I	07	5.38 ± 1.41	83.88 ±37.3
5. Stage II	11	9.92 ± 8.08	98.00 ± 45.1
6. Stage III	09	10.22 ± 3.35	148.78 ± 59.0
7. Stage IV	10	14.42 ± 4.27	274.33 ± 82.6

Non-irradiated: P <0.05: [3 vs 1,2] [7 vs 1,4]
Irradiated: P <0.05: [3 vs 1,2] [7 vs 1,4,5,6] [6 vs 1] [5 vs 1]

Table 3: Binucleated peripheral blood lymphocytes with Two Micronuclei /1000 Binucleated PBLs scored per subjects on exposure to γ-rays [2 Gy]

Group	Sample size [n]	Non-irradiated mean± SD	Irradiated mean± SD
1. Control	10	0.60 ± 0.84	3.40 ± 3.72
2.Fibroadenoma	29	0.35 ± 0.66	5.48 ± 3.08
3. Breast cancer [pooled from all the stages]	37	1.41 ± 2.01	13.98 ± 6.08
Breast cancer			
4. Stage I	07	0.25 ± 0.46	9.75 ± 4.20
5. Stage II	11	0.58 ± 1.00	11.75 ± 4.29
6. Stage III	09	0.89 ± 1.17	16.22 ± 7.45
7. Stage IV	10	3.42 ± 2.50	17.33 ± 5.45

Non-irradiated: P <0.05: [3 v/s 2] [7 v/s 1,4,5,6]
Irradiated: P <0.05: [3 v/s 1,2] [7 v/s 1,4] [6 v/s 1] [5 v/s 1]

Table 4: Binucleated peripheral blood lymphocytes with Three Micronuclei /1000 Binucleated PBLs scored per subjects on exposure to γ-rays [2 Gy]

Group	Sample size [n]	Non-irradiated mean± SD	Irradiated mean± SD
1. Control	10	0.00 ± 0.00	0.10 ± 0.32
2.Fibroadenoma	29	0.03 ± 0.18	1.19 ± 1.51
3. Breast cancer [pooled from all stages]	37	0.02 ± 0.16	3.66 ± 2.83
Breast cancer			
4. Stage I	07	0.00± 0.00	1.00 ± 1.20
5. Stage II	11	0.00 ± 0.00	2.58 ± 1.88
6. Stage III	09	0.00 ± 0.00	4.56 ± 2.13
7. Stage IV	10	0.08 ± 0.29	5.83 ± 3.04

Irradiated: P <0.05: [3 vs 1,2] [7 vs 1,4,5] [6 vs 1,4]

Table 5: Binucleated peripheral blood lymphocytes with Four Micronuclei /1000 Binucleated PBLs scored per subjects on exposure to γ-rays [2 Gy]

Group	Sample size [n]	Non-irradiated mean± SD	Irradiated mean± SD
1. Control	10	0.00 ± 0.00	0.00 ± 0.00
2. Fibroadenoma	29	0.00 ± 0.00	0.00 ± 0.00
3. Breast cancer [pooled from all the stages]	37	0.00 ± 0.00	1.56 ± 1.87
Breast cancer			
4. Stage I	07	0.00 ± 0.00	0.13 ± 0.35
5. Stage II	11	0.00 ± 0.00	0.83 ± 1.11
6. Stage III	09	0.00 ± 0.00	1.67 ± 1.66
7. Stage IV	10	0.00 ± 0.00	3.17 ± 2.12

Irradiated: P <0.05: [3 vs 1,2] [7 vs 1,4,5]

Table 6: Total number of Micronuclei /1000 Binucleated PBLs scored per subjects on exposure to γ-rays [2 Gy]

Group	Sample size [n]	Non-irradiated mean± SD	Irradiated mean± SD
1. Control	10	5.60 ± 2.50	26.40 ± 8.87
2. Fibroadenoma	29	6.19 ± 2.70	66.71 ± 23.60
3. Breast cancer [pooled from all the stages]	37	13.39 ± 9.01	203.70 ± 118.6
Breast cancer			
4. Stage I	07	5.88 ± 2.03	106.88 ± 45.63
5. Stage II	11	11.50 ± 9.68	133.42 ± 55.48
6. Stage III	09	11.78 ± 3.63	201.56 ± 81.06
7. Stage IV	10	21.50 ± 8.39	340.17 ± 97.58

Non-irradiated: p<0.05: [3 v/s 1,2] [7 v/s 1,4,5,6]
Irradiated: P <0.05: [3 v/s 1,2] [7 v/s 1,4,5,6] [6 v/s 1] [5 v/s 1]

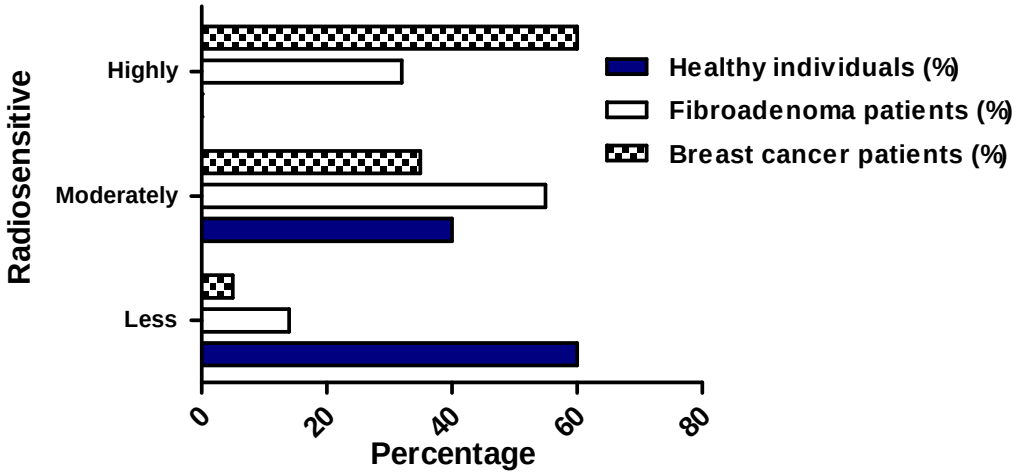

Figure 1: Distribution of subjects on the basis of their radiosensitivity

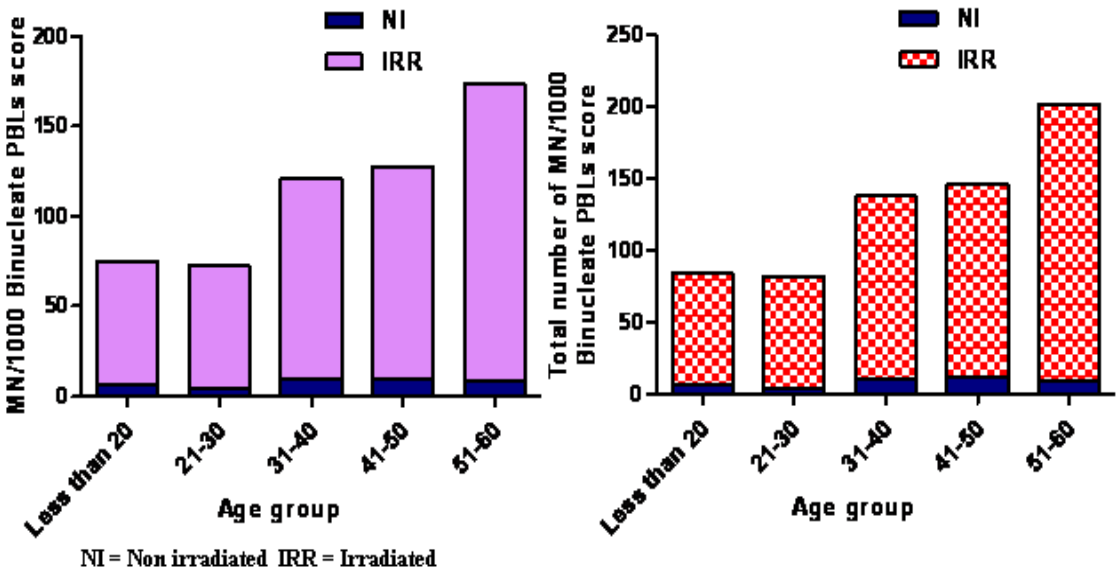

Figure 2: Influence of age on MN frequency in subjects [control, fibroadenoma and breast cancer patients] exposed to radiation [γ-rays, 2 Gy]

The study was conducted on blood samples obtained from 76 subjects. Of these the control set consisted of 10 individuals [13.2%], 29 patients with fibroadenoma [38.2%] and 37 patients [48.6%] suffering from breast cancer. Breast cancer patients were further grouped based on clinical stage of the cancer and stage I consisted of 8 [18.9%], stage II of 11 [29.7%], stage III of 9 [24.3%] and stage IV included 10 [27.1%] patients. Thirty four subjects [45%] tested were over 40 years of age. Those less than 20 years were only two [<3%] and individuals in the age group of 21-40 years were forty in number [>52%].

In non-irradiated samples the binucleated PBLs with MN [Table 1], showed an increase in fibroadenoma and breast cancer samples as compared to controls. In fibroadenoma samples the frequency of PBLs with micronuclei increased marginally [i.e 1.1 times], whereas in breast cancer the degree of increase was 2.1 times that of control [P<0.05]. Subsequent to radiation, the PBLs with MN increased 4.5 times in control, 10.4 times in fibroadenoma and 15 times in breast cancer samples as compared to corresponding samples without radiation exposure. Thus in breast cancer samples the degree of increase was 3.3 times more in comparison to control and 1.4 times more in

comparison to fibroadenoma. Further the homogeneity of variance in non-irradiated or irradiated samples showed significant correlation [$P<0.01$], thus indicating high inter individual variation. Further a stage wise increase in frequency of PBLs with MN, stage I to stage IV, both in non-irradiated and irradiated samples of breast cancer. In comparison to stage I, stage IV showed 3.2 times more PBLs with MN [$P<0.05$].

Frequency of PBLs with one micronuclei [Table 2] increased 4.4 times in control, 10 times in fibroadenoma and 15 times in breast cancer samples as compared to their corresponding samples without radiation exposure. Thus, the degree of increase was highest in breast cancer and lowest in control. Further from stage I to IV the frequency of PBLs with one micronuclei increased 2.7 times in non-irradiated and 3.3 times in irradiated samples [$P< 0.05$].

Frequency of PBLs with two micronuclei [Table 3] was nearly 2.4 times more in breast cancer patients as compared to controls. Stage wise there was no regular correlation. On irradiation highest degree of increase in the frequency of PBLs with two micronuclei, was observed in fibroadenoma [15.7 times], followed by breast cancer [9.9 times] and controls [5.7 times] as compared to their corresponding samples without radiation exposure. Further, from stage I to stage IV, the frequency of PBLs with two micronuclei increased 1.8 times [$P<0.05$] and this increase was regularly observed between different stages.

In non-irradiated control samples, no PBLs with three micronuclei was found. No of PBLs with three MN was insigninificant in fibroadnoma and breast cancer samples also. However in irradiated samples the degree of increase was maximum [182 times] in breast cancer and minimum in control [0.1 times] as compared to their corresponding samples without radiation [$P<0.05$]. Further the frequency of cells with three micronuclei increased 5.8 times between stage I and stage IV [$P<0.05$].

In non-irradiated samples no PBLs with four nuclei [Table 5] was observed in controls as well as fibroadenoma and breast cancer samples. However on radiation exposure , only breast cancer samples showed micronuclei, and stage wise comparison revealed a gradual stage matched increase in micronuclei frequency from stage I to stage IV [$P<0.05$]. The degree of increase in total number of MN frequency [Table 6] was 4.7 times in control, 10.8 times in fibroadenoma, 15.2 times in breast cancer samples as compared to their corresponding non-irradiated samples. Stage wise comparison showed gradual increase from stage I to stage IV in both non-irradiated and irradiated breast cancer samples [$P<0.05$].

Data from the study of PBLs from fibroaenoma patients showed majority of patients i.e., 55.2% moderately radiosensitive, while 31.3% patients highly radiosensitive, while 13.8% patients were radio resistant to the radiation induced genomic damage at 2 Gy level [Figure 1]. Data from the breast cancer patients indicated that majority of cases i.e., 59.5% were highly radiosensitive, 35.1% were moderately radiosensitive, and 5.4% patients were radio resistant. To evaluate the influence of age on MN frequency individuals of various age groups [<20y; 21-30y; 31-40y; 41-50y; 51-60y] were examined [Figure 2]. It was found that patient's age did not influence MN frequency in both non-irradiated and irradiated samples [$P< 0.05$].

DISCUSSION

Patients with fibroadenoma have not been studied with the MNT so far. Determining the acceptable level of genetic damage in a human population is a health need. The exact mechanism of MN formation are still not completely understood, it is well known that radiation induced MN arise mainly from acentric chromosomal fragments though a significant minority [18]. An individual can be investigated in vitro [after X-irradiation] and in vivo [during radiotherapy] is a good biological tool for developing assays that are able to envisage individual radiotherapy reaction.

In the present work, the specific culture conditions like serum concentration, length of incubation and harvesting, fixation and slide preparation, were standardized to obtain the best yield of binucleate cells from non-irradiated and gamma irradiated blood. MN score in lymphocytes depends on the proportion of cells that have been transformed due to mitogenic exposure with further commitment for cell division, fraction of the cells not divided and cell which has divided more than once. To overcome this problem, Fenech and Morley [19] used Cytochalasin B to block cytokinesis. With this approach, MN can be scored only in the dividing binucleate cells. The cytokinesis blocking method of Fenech and Morley [19] is adopted in the present study with minor modifications. In this study, optimum

concentrations of cytochalasin B was 4 µg/ml and before harvesting the cells were incubated in hypotonic solution KCl for 5 minutes.

Size of MN in human lymphocytes found to vary according to the inducing agent used. In the present study, size measurements of MN were not attempted, and also comparative study with different inducing agent was not carried out. The effect of intrinsic variables like age, on the frequency of micronuclei is still unsolved. In the present study, a distinct pattern between the age group and the frequency of cells with micronuclei could not be observed as the data showed a non-uniform variation. Similar observation was found when the overall micronuclei frequency was compared vis a vis the age group which was also statistically not significant [$P>0.05$] which is similar to the earlier report [15], suggesting a non contributory role of age in determining MN frequency.

Spontaneous or baseline MN frequencies in cultured lymphocytes provide an index of accumulated genetic damage occurring during the life span of circulating lymphocytes [19]. The aim of the study was to evaluate intrinsic radiosensitivity of lymphocytes in patients with benign, malignant breast disease and healthy donors. Binucleated PBLs with MN significantly increased in irradiated as well as non-irradiated breast cancer samples in comparison to control and fibroadenoma samples. Further total number of MN also showed significant increase in irradiated and non-irradiated breast cancer samples in comparison to control and fibroadenoma samples. With the increasing stage of the cancer, increased MN frequency was significant. All the above findings indicate the usefulness of MN assay in assessing the degree of genetic damage as well as radiosensitivity. The comparison of MN scores between in fine needle aspirations cytology smear of fibroadenoma and infiltrating ductal carcinoma and between various grades of infiltrating ductal carcinoma reported to be significantly different [13]. Suggestive of the usefulness of MN assay, if it is done in peripheral blood lymphocytes, more advantageous as it is noninvasive, permits the investigator to access the tissue repeatedly throughout a trial as compared to biopsies or using markers. Lymphocyte radiosensitivity measured before and after in-vitro X-irradiation along with DNA repair ability might give a complete cytogenetic picture of individual response.

It also intended to investigate the range of lymphocyte radiosensitivity among and between healthy donors, benign and malignant breast disease patients. Variable inter- individual response to radiation induced MN suggest that, individual's radiosensitivity might play a role during radiation treatment. Knowledge of a patient's individual radiosensitivity before radiotherapy could help to plan the most appropriate clinical treatment [radiotherapy, chemotherapy, surgery or combination]. The present work shows large variation in inter-individual radiosensitivity of patients [MN mean13.3 ± 9.01 S.D] and thus offering increased possibility to identify radiosensitive patients on the basis of MN frequency. On the contrary healthy donors presented fewer variations in distribution of MN count [mean 5.6 ± 2.5 SD] enabling a reliable cut off level that helped to distinguish radio resistant from radiosensitive individuals, thus supporting earlier findings [20]. Contradictory results have also been published by some researchers [21, 22], who did not find significant differences in the MN number either in the baseline or in irradiated cells.

The implication of the present study is that if the lymphocytic radiosensitivity can be reliably assessed in-vitro, the in-vivo dose on radiation treatment can be suitably modified, just enough to destroy the cancer tissue, not harming the normal tissues. There is much evidence supporting the concept of inter-individual variation in normal tissue radiosensitivity. It is believed that 15% of the patients receiving radiation treatment may be radiosensitive [22]. There are considerable interests in developing methods which can assess in-vitro radiosensitivity of normal fibroblasts or lymphocytes and be useful as a predictive assay for normal tissue response to radiotherapy. Micronuclei assay can be used for normal cells to determine the intrinsic radiosensitivity of individuals [23]. Correlation between the intrinsic radiosensitivity of fibroblasts and late effects has been reported for cancer patients [24]. In comparison with the fibroblasts, lymphocytes seem to be more attractive for measuring normal tissue radiosensitivity because they can be easily obtained and cultured in-vitro. Further, lymphocytes from those breast cancer patients who showed severe reactions to radiotherapy are more sensitive than those from normal donors [25]. As enhanced sensitivity to the chromosome damaging effects of ionizing radiation is a feature of many cancer-predisposing conditions, studies on comparison between BRCA1 mutation and MN frequencies, supports the usefulness of induced MN as a biomarker for cancer predisposition and suggest its implication as a screening test for carriers of a BRCA1 mutation in breast cancer families [26].

As carcinogenicity of sex hormones is considered to be the result of a combination of genotoxic and epigenetic modes of action, study reported increased formation of MN after hormonal stimulation was not due to the

chromosomal damage [27]. Contradictory findings were reported from Geara [28], who did not find any correlation between lymphocyte radiosensitivity and acute or late effects of radiation for head and neck cancer patients. There are only few reports on difference in radosensitivity between healthy donors and cancer patients assessed by MN assay. Data from the present study indicate that majority of breast cancer cases [59.5 %] were "highly radiosensitive" whereas 31.5 % patients were "moderately radiosensitive" and a minority of patients [5.4%] were radio resistant. Our results are in agreement with the earlier observations [15, 29] who found increased radiosensitivity in cancer patients vis a vis their micronuclei frequency. However, Ochi Lohmann [30] reported insignificant difference in the frequency of MN between normal subjects and cancer patients. Comparison of radiosensitive and radio resistant patients has also revealed a difference of MN at 4 Gy and not 2 Gy [31]. Thus it is possible that by using higher dose levels and low dose rate, cellular radiosensitivity assessment may provide a better discrimination between individuals [32].

In the present study, "radiosensitive" and "radio resistant" is arbitrary. Based on the data from control subjects, a pattern has been formulated which enabled better analysis of data from fibroadenoma and cancer patients. However individual opinion about the exact "cut off level" may vary, which may result in decreased proportion of patients belonging to the "radio resistant" category, but from the data, the numerical count of patients in the "highly radiosensitive" group is unlikely to vary, as it has been placed at a significantly higher "cut-off" level. In fibroadenoma patients, majority of them [55%] were "moderately radiosensitive", 32% were highly radiosensitive and a small proportion of patients [13%] were radio resistant. However, majority of control subjects were radio resistant [60%] and rest were moderately radiosensitive. High radio sensitivity in the lymphocytes from breast cancer patients has been reported in the past [33].

In the present study, the dose response relationships were fitted to linear model. The aim of the study was not only to establish a dose response relationship between gamma ray doses and MN in human peripheral blood lymphocytes in multiple donors, but also to validate the test system. In some cells, the non-linear dose response may be observed as result of saturation of DNA repair at low doses [34]. At low dose exposure, damaged cells may not contain more than one fragment of chromosome, which will have very small probability of becoming MN. Findings suggest that MN yield in human PBLs offers a reliable acute and perhaps chronic biodosimeter for in-vivo radiation dose estimation.

Antioxidant status in breast cancer patients of different ages after radiotherapy indicated response to radiotherapy involves age related impairment of antioxidant capacity for elimination of H_2O_2 causing oxidative damage to blood cells, thus suggesting that cytotoxic effects of radiation on healthy tissues might be more pronounced during the aging of breast cancer patients, and should be considered in the further development of individualization protocols in cancer radiotherapy [35].

CONCLUSION

Importance:
The MN assay in cytochalasin blocked lymphocytes is a convenient tool for the assessment of genetic damage induced by radiation or chemical agents. It seems possible to use the yield of MN in human peripheral blood lymphocytes as a biological dosimeter in the field of radiation protection and probably for low doses also. All results were best fitted to the linear model thus differentiating the observations. The assay is also cost effective and statistically more powerful and more precise than traditional metaphase scoring analysis of chromosome aberration or sister- chromatid exchange [Heddle et al. 1983]. MN test also helps in detecting a eugenic activity [Heddle et al. 1983]. Based on its sensitivity, the micronucleus test has been recommended by the OECD [1983] and EEC [1984] as the minimum requirement for testing new pharmaceutical products.

Limitation:
A significant problem in the MN assay is the large variability of binucleate cell formation, i.e the size of scrabble cell population which depends on the degree of lymphocyte proliferation. MN assays can only be effective as quantitative biological dosimeters if one can identify those cells, which have divided once after exposure, because only dividing cells can express micronuclei. The main difficulty in using the assay has been time consuming procedure to score the preparations.

Future:
If the blood samples of the patients prior to specific therapy analyzed for blood cell tolerance and perhaps normal tissue tolerance, it might be possible to escalate the dose to the remaining patients to improve the local control and cure rates in other groups of patients undergoing same radiotherapy. Thus, an in-vitro cytogenetic test applied to lymphocytes before radiotherapy, which can indicate their behavior during radiotherapy is a stimulating idea in the field of predictive assays. Lymphocytes are easy to obtain and culture. Also, the results are available in a short time [less than one week] compared with the 2-3 months necessary for the colony formation assay on fibroblast or tumour cells.

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