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Chromosomal Sensitivity to Ionising Radiation in Lymphocytes of Patients with Head and Neck Cancer

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ABSTRACT

Background: The aim of this study was to test the in vitro sensitivity of lymphocytes of patients with head and neck cancer against gamma irradiation and also to find out if the frequencies of chromosomal aberrations correlate with side effects of radiotherapy.

Methods: Peripheral blood of 101 patients with head and neck cancer was collected before the onset of radiotherapy, cultured and irradiated in the G-2 or the G-0 phase of the cell cycle. Lymphocytes of 40 healthy donors were treated in the same way. Chromosomal aberrations such as chromosome and chromatid breakages, chromosome and chromatid gaps, chromatid exchanges and micronuclei were scored in metaphase cells of the patient and control groups.

Results: The frequency of radiation- induced G2 aberrations in lymphocytes of patients were on average higher than that of healthy donors (P=0.001 for chromosomal breaks). The frequency of radiation-induced micronuclei in the G0 assay were also higher in patients than that in controls (P=0.05). The results also indicate that there is no correlation between the two assays. No significant correlation was observed between aberration frequencies in lymphocytes and the degree of both early and late normal tissue reactions.

Conclusion: The induced chromosomal aberration frequencies in lymphocytes of patients with head and neck cancer are higher than those in healthy individuals, however, it does not appear to have a predictive value for the risk of developing side effects to radiotherapy.

Keywords: Head and neck cancer, gamma irradiation, G2 assay, micronuclei assay.

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Introduction

Head and neck cancer (HNC), defined as cancers of Para nasal sinuses, nasal cavity, oral cavity, pharynx, and larynx. HNC is the sixth most common type of cancer, representing about 6% of all cases, with 650, 000 new cases and 350,000 cancer deaths are estimated to occur each year word-wide[1-3]. Early diagnosis of this cancer provides the best predictor of survival[4].

Almost all of these epithelial malignancies are squamous cell carcinomas that arise from the upper aero-digestive tract[5]. Exposure to alcohol and tobacco smoking are the major risk factors for HNC[6,7]), however, a subgroup of patients develops squamous cell carcinoma of the head and neck in which the role of tobacco or alcohol use as a risk factor is less clear in the etiology of their cancer[8]. Other important risk factors include human papilloma virus infection[9,10] and genetic susceptibility[11,12]. A few epidemiologic studies reported elevated risks of HNC or oral and pharyngeal cancer in relatives of affected individuals[13,14]. These reports provide evidence that genetic factors contribute to HNC susceptibility.

It is suggested that higher frequency of chromosomal damage in peripheral blood lymphocytes of individuals is a marker of cancer predisposition[15]. The results of a few studies also indicate that an enhanced radio sensitivity of human peripheral blood lymphocytes is associated with overall cancer risk[16-18]. Support for this hypothesis comes from the demonstration that many inherited cancer-prone diseases such as ataxia-telangiectasia, Nijmegen breakage syndrome and hereditary retinoblastoma show evidence of this type of elevated radio sensitivity[19-21].

Concerning various head and neck cancer patients, several studies have evaluated the association between frequency of radiation-induced chromosomal aberrations and the risk of HNC cancer but the results have been inconsistent[22-24] . In a study conducted by Lisowska and co-workers, they observed enhanced radiation-induced chromosomal aberration in larynx cancer patients[22], but in a large case-control study of patients with head and neck cancer at different anatomical tumor sites, chromosomal sensitivity to radiation of larynx cancer patients did not differ from that in controls[24]. Braakhuis and colleagues did not observe any evidence of chromosomal damage in lymphocyte of young adults with oral squamous cell carcinoma compared with those in healthy controls[25].

This study was undertaken as it has been suggested that genetic factors is particularly important in patients with head and neck cancers, where there will be a reduced effect of cumulative environmental factors[26]. In the present study, we used two chromosomal radio-sensitivity assays, the G2 and the G0-micronucleus assays. The G2 assay involves the analysis of chromosome damage during G2 phase of the cell cycle, whereas in the Go assay, chromosome deletions as micronuclei will be detected in post mitotic cells.

Methods

1. Patients and Controls

In the present study a total of 141 subjects, consisting of 101 histo-pathologically confirmed head and neck cancer patients and 40 healthy control individuals were recruited as study subjects. Head and neck cancer patients and the control group were consecutively recruited between April 2012 and February 2015 from Clinics of Cancer Institute, Tehran University of Medical Sciences. Diagnosis of head and neck cancer was based on pathological examination of the tissue or biopsies of the tumor. The selection criteria for the patients were no history of autoimmune diseases and patients who suffered diseases such as diabetes mellitus or thyroid disorders were not included in this study. The control

group consisted of gender and age matched healthy individuals recruited from the same hospital as that of the cases. The selection criteria for the controls were no evidence of family history of any cancer or other chronic disease. This study was approved by the Ethical Committee of Tehran University of Medical Sciences, and written informed consent was obtained from the subjects for blood sampling and interview.

2. Cytogenetic Methods

2.1. Experimental Protocol for G2 Assay: G2 assay was performed essentially as described by Scott et al[27] with minor modification. Prior to culturing, heparinized blood samples from all participants were kept 4 hours at 26°C. For each blood sample, two tissue culture flasks were set up, one for in vitro irradiation, the other served as control (un-irradiated) for analysis of the spontaneous chromosomal aberrations. To each flask, 0.5 ml of the blood was added in 4.5 RPMI-1640 culture medium supplemented with 10% fetal calf serum, 1% L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. Phytohemagglutinin (PHA) at final concentration of 1 µg/ml was used to induce lymphocyte proliferation. The flasks were incubated in a humidified air atmosphere at 37°C with 5% CO_2 for 4 days. Four hours before harvesting, the cultures were exposed to gamma irradiation (Theratron 780e, MDS, Canada; 60Co, 70cGy/min) with a dose of 100 cGy at room temperature. After 2 more hrs. incubation, colcemid (Gibco, final concentration 0.15 µg/ml in the medium) was added to arrest the cells at metaphase. Materials of each flask were transferred to a centrifuge tube, and then centrifuged at 1200 RPM for 10 minutes to harvest lymphocytes. The supernatant was then removed and cells were treated with 5 ml of 0.075 M KCl for 15 minute. After further centrifugation, the KCl was removed and the cell suspensions were fixed with fresh fixative (methanol/glacial acetic acid; 3/1) and this process was performed two more times. The cells in suspension were dropped on to clean coded slides. The slides were dried in air and stained with 2% Giemsa (in phosphate buffer saline, pH 7.0) for 5 minutes. For structural chromosome aberration study, 100 metaphases from both exposed and control flasks were scored for aberrations such as chromosome and chromatid breakages, chromosome and chromatid gaps, chromatid exchanges and fragmentations.

2.2. Experimental Protocol for G0-micronucleus Assay: Full details are given elsewhere[28]. Briefly, two tissue culture flasks were prepared and set. One of the flasks of each donor was exposed to gamma rays uniformly, total dose 300 cGy (Theratron 780e, MDS, Canada; 60Co, 70cGy/min) at room temperature. Lymphocytes were stimulated to proliferate with PHA (final concentration 1 µg/ml). The flasks were incubated at 37°C (with 5% CO_2). Forty hours later, cytochalasin B (Sigma) was added with a final concentration of 4 µg/ml. After further incubation, cells were harvested at 90 hs post-stimulation by hypotonic shock with 0.075 M KCl, followed by fixation, three times, in methanol/acetic acid (3:1) solution. Slides were stained in 2% Giemsa for 5 min. For analysis, slides were coded and investigated blindly. Per slide 500 binucleated cells (BNCs) were scored for micronucleus (MN) frequencies. The number of BNCs containing two or three MN was also recorded.

3. Statistical analysis

The data were analyzed using the SPSS 16.0 program for Windows (SPSS Inc., Chicago, IL, USA). For each patient or control individual, frequency of chromatid or chromosome type aberrations in 100 metaphases were calculated as well as micronuclei per 500 binucleated lymphocytes. The results were recorded as mean and standard deviation in 100 metaphases for each group and then statistically

analyzed using Student’s t-test. $P<0.05$ was considered statistically significant.

Results

The patient and control groups were comparable in age and sex. The mean age of patients was 56.35±13.5 years and of control group was 43.97±9.1. The mean number of each chromatid or chromosome type aberrations per 100 metaphases of patients compared to those in healthy individuals are presented in Tables 1 and 2.

Our results showed that the mean number of

Table 1. The mean number of chromosomal aberrations per metaphase in peripheral blood lymphocytes of head and neck cancer patients compared with those in healthy individuals.

Groups	Chromatid breaks/cell (Mean±s.d.)	P-value[a]	Chromosome breaks/cell (Mean±s.d.)	P-value[a]	Chromosome aberrations/cell (Mean±s.d.)	P-value[a]
Group 1* (n=101)	0.16±0.09	0.003	0.11±0.04	0.001	0.27±0.13	0.001
Group 2* (n=40)	0.13±0.03		0.07±0.02		0.20±0.03	

Independent Samples T test. *Group 1: Head and neck cancer patients, *Group2: Control subjects.

Table 2. The mean number of micronuclei and chromosomal aberrations per metaphase in peripheral blood lymphocytes of head and neck cancer patients compared with those in healthy individuals.

Groups	Chromatid gap/cell (Mean±s.d.)	P-value[a]	Micronucleus/cell (Mean±s.d.)	P-value[a]	Fragments	P-value[a]	Rearrange/cell[b]	P-value[a]
Group 1* (n=101)	0.21±0.13	0.02	0.024±0.016	0.05	0.20±0.38	0.02	0.005±0.02	0.08
Group 2* (n=40)	0.16±0.03		0.019±0.003		0.06±0.22		0.001±0.001	

a, Independent Samples T test, b chromosomal rearrangements/cell.

*Group 1: head and neck cancer patients, Group 2: Healthy individuals.

abnormalities including chromatid breaks (P=0.003), chromosomal breaks (P=0.001), total Chromosomal aberrations (P=0.001), micronuclei (P=0.05), chromatid gaps (P=0.02) and fragments (P=0.02) are significantly higher in patients with head and neck cancer compared with those in control group.

Discussion

In the present study, we investigated the in vitro G2 sensitivity of lymphocytes to radiation of patients with head and neck cancer against gamma irradiation and also we analyzed if the frequencies of chromosomal aberrations in these patients correlate with side effects of radiotherapy. The results show that head and neck cancer patients have a significantly higher mean number of radiation-induced chromosomal aberrations compared with those in control group. We did not detect any correlation between the frequency of radiation-induced chromosomal aberrations and the degrees of tissue reactions.

There are some published data regarding the in vitro sensitivity of lymphocytes to radiation in patients with different cancer but the results are somewhat inconsistent.

A study was performed by Papworth and co-workers who analyzed the G2 radiosensitivity in peripheral blood lymphocytes of 42 patients with head and neck cancer, 16 patients before therapy and 26 post therapy patients. The results were compared with a group of 27 healthy controls. The mean frequency of radiation-induced G2 aberrations was higher in the 42 patients than in 27 normal controls, but not significantly. However, cases less than 45 years old were significantly more sensitive than normal of the same age range. This finding is consistent with our results, but the study performed by us was of a prospective type and blood was collected before the onset of therapy[29]. With the micronuclei assay, we found that the mean frequency of induced micronuclei for patients was higher than that of the controls, however on the borderline of significance (P = 0.05, Table 2). Similar result is reported by Burgaz and his colleagues[30]. The results of the present study are also in agreement with the findings of Terzoudi et al[31] and Lisowska et al[22], who both reported enhanced chromosomal radiosensitivity in larynx cancer patients. This increase was statistically significant in the study of Lisowska et al, but in the study of Terzoudi and his colleagues, the statistical significance was not stated. Gajecka et al. also demonstrated that in a group of 52 patients with laryngeal cancer, chromatid breaks per cell were almost two-fold higher compared to 47 healthy controls[32].

In conclusion, the present study indicates that the frequency of radiation-induced chromosomal aberrations, G2 and micronuclei, in peripheral blood lymphocytes of patients with head and neck cancer was higher compared with the healthy individuals. We did not detect any correlation between the frequency of radiation-induced chromosomal aberrations and the degrees of tissue reactions.

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Conflicts of Interests

none to declare.

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