**ABSTRACT**

**Background:** Acute lymphoblastic leukemia (ALL) constitutes a heterogeneous group of diseases characterized by abnormal proliferation and accumulation of immature lymphoblasts arrested at various differentiation stages. Increasing evidence suggests that chromosomal defects are present in these patients. The aim of this study is to investigate the chromosomal radiosensitivity in a group of ALL patients.

**Materials and Methods:** To analyze chromosomal radiosensitivity of ALL patients, lymphocytes of 20 patients were cultured followed by exposure to γ-ray irradiation to detect the chromosomal aberrations as an indicator of radiosensitivity. Cells were scored for the number of aberrations (chromatid breaks, chromatid gaps, chromosome breaks, chromosome gaps and chromatid exchanges). Results were compared with healthy individuals, and ataxia telangiectasia (AT) patients as positive control.

**Results:** On average number of aberrations in ALL patients was significantly higher than that in healthy controls. We found 65% of ALL patients appeared to be susceptible to in vitro irradiation. Chromosomal radiosensitivity of 35% patients was not different from healthy donors. Ataxia telangiectasia patients showed the highest degree of lymphocyte radiosensitivity. The results also indicate that there is a good correlation between the two assays, G2 and G0, using the same blood sample for both assays.

**Conclusion:** According to the result, we concluded that most of the ALL patients are sensitive to ionizing radiation and therefore should be protected from unnecessary diagnostic and therapeutic procedures using ionizing radiation.

**Keywords:** Chromosomal sensitivity, gamma irradiation, acute lymphoblastic leukemia
Acutelymphoblastic leukemia (ALL) comprises a biologically and clinically heterogeneous group of diseases. This disease shows variations with respect to morphological, cytogenetic, and immunologic features of the transformed cells, that results in a diverse clinical behavior and different responses to therapy. It is manifested by clonal proliferation, decreased apoptosis and the malignant proliferation and accumulation of immature lymphoid cells that are arrested at various differentiation stages within the bone marrow and lymphoid tissues.

ALL patients usually have high white blood cell counts and may present with organomegaly, particularly mediastinal enlargement and CNS involvement. ALL is the most common form of childhood malignancies but it can affect all age groups and constitutes 13% of acute leukemia in adults with slightly higher frequency in men than women.

The etiology of ALL is unknown; however, Ionizing radiation especially in high doses and with acute exposure has been suggested as a possible risk factor in leukemogenesis. In some studies, chromosomal defects and molecular abnormalities have been consistently observed in ALL patients.

Furthermore, enhanced chromosomal radiosensitivity and cancer-prone genetic disease such as ataxia-telangiectasia and Nijmegen breakage syndrome has also been found in ALL patients. These patients do not tolerate conventional dose of radiation therapy.

Most studies performed on ALL, have focused on classifying patients into various risk groups based on known prognostic and predictive factors. The risk of severe reactions resulting from radiotherapy limit the total dose prescribed for patients, so it is important to identify these radiosensitive patients to avoid reactions and to apply less invasive strategies in these patients.

The current study was undertaken to investigate the chromosomal radiosensitivity by means of the G2 assay and the G0-micronucleus (MN) assay in a group of ALL patients. As a positive control we used ataxia telangiectasia (AT) patients and compared the results both with AT patients and healthy individuals as control.

A. Patients and controls:
Between March 2009 and June 2010, twenty consecutive patients with ALL, who had been referred to the Children’s Medical Center Hospital, the main referral center for pediatric leukemia in Iran, took part in this study. Diagnosis of ALL was based on morphologic and immunophenotypic criteria. Clinical and laboratory data of the patients were documented. Thirty age-matched healthy individuals were randomly selected as control group. This study was reviewed and approved by the Ethics Committee of the Faculty of Medicine in Tehran University of Medical Sciences. After taking informed consent from the patients, venous peripheral blood samples were obtained stored in lithium heparin blood tubes. Blood cultures were started within 4 hours of collection.

B. Methods

1. The G2 assay: The assay was performed as described by Scott et al. Briefly, heparinized blood was kept at room temperature prior to culturing, which was within 4 hours of the blood collection. For each sample, two tissue culture flasks (25 cm2) were set up: one for in vitro irradiation, the other served as control (un-irradiated). To each flask 0.5 ml of the blood was added to 4.5 complete RPMI-1640 culture medium supplemented with 10% fetal calf serum, 1% L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. The lymphocytes were stimulated to proliferate with 1% phytohemagglutinin (PHA, Invitrogen, final concentration 1 µg/ml). The flasks were incubated in a humidified air atmosphere at 37°C with 5% CO2 for 4 days. After incubation, the culture lymphocytes were exposed to gamma irradiation (Theratron 780e, MDS, Canada; 60Co, 70cGy/min) with a dose of 100 cGy at ambient temperature. After 2h post-irradiation, 0.2 ml Colcemid (Gibco, final concentration 0.1 µg/ml medium) was added to block cells at metaphase. Lymphocytes were harvested by centrifugation of the contents of each flask, supernatant was removed and for hypotonic shock, cell pellets were re-suspended in 5 ml of 0.075 M KCI for 15 min on ice. After further centrifugation, supernatant was again removed and cells were fixed three times in fresh fixative (methanol/glacial...
acetic acid, 3/1). For making slides, cells were dropped on clean slides. Slides were stained with 2% Giemsa (in phosphate buffer) for 5 minutes. One hundred well-spread metaphases were analyzed from both irradiated and control samples and scored for aberrations such as chromatid breaks, chromatid gaps, chromosome breaks and chromatid exchange. Frequency of aberrations in control samples was subtracted from that in irradiated samples to give the induced yield.

2. The G0-micronucleus (MN) assay: Full details of this assay are given elsewhere. Briefly, heparinized blood samples were stored at room temperature for 4 hours. Two tissue culture flasks (25 cm²) were set up: one for in vitro irradiation, the other served as control (unirradiated). An aliquot of 0.5 ml of the blood was diluted with complete RPMI-1640 culture medium in the ratio of 1:9. Complete culture medium was supplemented with 10% fetal calf serum, 1% L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. One of the flasks of each donor was irradiated, total dose 3 Gy (source: Theratron 780e, MDS, Canada; 60Co, 70cGy/min) at ambient temperature. After irradiation lymphocytes were stimulated with 1% PHA (final concentration 1 µg/ml). Flasks were incubated at 37°C (with 5% CO2). Forty-four hours later, cytochalasin B (Sigma) was added to a final concentration of 6 µg/ml. After further incubation, cells were harvested at 92 h post-stimulation by hypotonic shock with 0.075 M KCl, followed by fixation, three times, in methanol/acetic acid (3:1) solution. For analysis, slides were coded and randomized. Per slide 500 binucleated cells (BNCs) were scored for micronucleus (MN) frequencies.

Statistical analysis
Statistical analysis of data was performed using SPSS statistical software package (version 16.0). Results were presented as the mean ± standard deviation. Independent-samples t-test was performed to compare means between the groups.

P-values of less than 0.05 were considered statistically significant.

Results
Mean number of chromosomal aberrations such as chromatid breaks, chromatid gaps, chromosome breaks, chromosome gaps and chromatid exchange for the ALL patients, the control healthy group and the positive AT patients are presented in Table 1. As shown, the mean number of any kind of aberrations, except chromosome breaks, in ALL patients was significantly higher than that in healthy controls. Besides, 65 percent of these patients appeared to be susceptible to in vitro irradiation. Chromosomal radiosensitivity of 35% patients was not different from healthy donors. Ataxia telangiectasia patients showed the highest degree of lymphocyte radiosensitivity than ALL patients and healthy individuals.

Discussion
Acute lymphoblastic leukemia is the most frequent cancer in childhood and is associated with good outcomes. Currently, 80% of children with ALL treated with anti-cancer therapies are alive and disease-free after 5 years of therapy completion. Recently, number of patients with second neoplasia has increased among long-term survivors of childhood ALL. There is also compelling evidence that specific exposure to radiation and chemotherapy are the risk of developing secondary malignancy.

In the current study, the chromosomal radiosensitivity by means of the G2 assay and the G0-micronucleus assay was measured in a group of ALL patients and the results were compared with those of healthy individuals and AT patients as a positive control group. We found that ALL patients have a significantly higher mean number of in vitro radiation-induced chromosomal aberrations than those in healthy individuals. Besides, with respect to the proportion of sensitive patients, 65 percent of these patients were sensitive to ionizing radiation.

The relevance of in vitro increased frequency of chromosomal aberrations in peripheral lymphocytes of patients exposed to gamma radiation as indicator of cancer risk is supported by several studies. Moreover, enhanced chromosomal radiosensitivity has been demonstrated in certain cancer-prone diseases such as ataxia telangiectasia, ligase IV deficiency, Nijmegen breakage syndrome, hereditary retinoblastoma and primary immunodeficiency disorders.

The elevated chromosomal radiosensitivity has also been reported in a significant proportion of cancer pa-
Patients such as cases with breast cancer, colorectal cancer and various head and neck cancer including patients with oral cavity, pharynx and larynx cancers that all show the in vitro increased mean number of radiation-induced chromosomal aberrations compared with the healthy subject group. There is no similar report on performing the in vitro cytogenetic analysis, by G2 and G0-MN chromosomal sensitivity assay, in patients with ALL to evaluate the long-term effects of radiation therapy, however, our data are in agreement with the enhanced chromosomal radiosensitivity observed in a group of the young breast cancer patients who showed the highest percentage of radiosensitive cases in the G2 assay as well as in the G0-MN assay.

In conclusion, our data show that ALL patients are more sensitive to ionizing radiation than normal individuals as indicated by the G2 and by G0-MN assay, therefore they should be protected from unnecessary diagnostic and therapeutic procedures using ionizing radiation. In addition, the results of this study indicate that a good correlation exists between G2 and G0 assay using the same blood sample for both assays.

### Table 1- Mean values of chromosomal aberrations per 100 metaphases scored in peripheral blood lymphocytes exposed to γ-gamma rays for the ALL patients, healthy control group and the positive ataxia telangiectasia (AT) patients

<table>
<thead>
<tr>
<th>Aberration</th>
<th>ALL patients Group I (N = 20)</th>
<th>Healthy controls Group II (N = 30)</th>
<th>AT patients Group III (N = 7)</th>
<th>P(I vs. II)</th>
<th>P(I vs. III)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromatid breaks</td>
<td>33.5±16.1</td>
<td>23.5±9.6</td>
<td>50.1±12.2</td>
<td>0.003</td>
<td>0.02</td>
</tr>
<tr>
<td>Chromatid gap</td>
<td>46.8±16.8</td>
<td>37.2±11.5</td>
<td>70.2±14.1</td>
<td>0.01</td>
<td>0.007</td>
</tr>
<tr>
<td>Chromosome breaks</td>
<td>17.1±12</td>
<td>18.3±10.7</td>
<td>33.1±7.4</td>
<td>0.19</td>
<td>0.1</td>
</tr>
<tr>
<td>Chromosome gap</td>
<td>23.7±15.1</td>
<td>16.6±7.3</td>
<td>19.3±7.5</td>
<td>0.01</td>
<td>0.35</td>
</tr>
<tr>
<td>Fragmentation</td>
<td>12.5±7.1</td>
<td>9.5±4.1</td>
<td>15.5±3.1</td>
<td>0.001</td>
<td>0.15</td>
</tr>
<tr>
<td>Rearrangement</td>
<td>4.8±4.5</td>
<td>2.5±3.6</td>
<td>6.8±5.5</td>
<td>0.03</td>
<td>0.44</td>
</tr>
<tr>
<td>Micronucleus</td>
<td>73.9±21.1</td>
<td>17.3±5.9</td>
<td>96.5±13.7</td>
<td>0.001</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Chromosomal aberrations consisted of chromatid breaks, chromatid gaps, chromosome breaks, chromosome gaps, fragmentation, chromatid exchange and micronucleus.

### References


