Chromosomal aberrations induced by radiotherapy in lymphocytes from patients with lung cancer

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Abstract: In this study we tried to define incidence and types of chromosomal aberrations (CAs) caused by radiotherapy (RT) in circulating lymphocytes from patients with lung cancer. For this purpose, we used cumulative dose–effect relationship, and correlate these data with statistical parameters. CAs were evaluated in terms of chromosome break, dicentric, ring and chromosome gap. Abnormal metaphase number (AMN) was also calculated. Chromosome studies were carried out in peripheral blood lymphocytes of 20 cancer patients receiving RT. Patients were treated with 10 Gy of gamma (γ) radiation during five wk(s). In all patients, a significant increase in AMN and frequency of CAs (e.g. chromosome break, dicentric, ring and chromosome gap) observed during the RT depend on cumulative radiation dose when compared to before RT, and this increase was statistically significant (p<0.05). The highest CAs frequency was observed at the end of fifth wk. Among the CAs, chromosome breaks have a high incidence. But no CAs and abnormal metaphase was observed in lymphocytes before RT. The present study showed that RT possess a significant effect in increasing of CAs and chromosome break, dicentric, ring and chromosome gap are very sensitive and useful biomarkers in the study of this effect. In other words, these CAs may be used as possible fingerprints of RT.

Keywords: Chromosomal aberrations, Gamma radiation, Lung cancer, Radiotherapy

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Introduction

It has been observed for many decades that ionizing radiation causes mutational events, DNA and chromosomal damage. Especially X and γ radiations are known to be human carcinogens based on sufficient evidence in humans (Testard et al., 1996). Different studies indicate that these radiations causes various damages in splenocytes, hepatocytes and lymphocytes (Noorizadeh et al., 2004), and they also activates protooncogenes that are involved in early signal transduction (Chen et al., 1998). Besides, these rays may be caused CAs such as breaks, dicentrics, tricentrics, rings, and numerous acenetic fragments. Plenty of the studies have shown that the frequency of CAs, like rings and double–minute chromosomes, was increased in persons exposed to radiation. CAs are caused by direct DNA breakage, replication of a damaged DNA template, inhibition of DNA synthesis and other mechanisms such as inhibition of enzymes relating to chromosome formation (Hayata, 2005). As a result, it may be occurred cancers such as breast, thyroid, lung and leukemia in related cell (Movafagh et al., 2007; Brenner and Sachs, 2006). All these observations directed researchers to understanding the mechanisms and origin of CAs (Natarajan, 2002)

The studies on the biological effects of ionising radiation are carried out in mammalian tissues such as rat testes, eggs of Ascaris, testes of grasshoppers, larval cells of amphibians, somatic and meiotic cells of plants (Natarajan, 2002). Nevertheless, the lymphocytes are predominant for cytogenetic analysis in human cells. Because, they circulate in the blood and so receive equal radiation dose (Testard et al., 1996).

Consequently, radiation is extensively used for cancer therapy, and chromosome analysis is a good tool with which to assess the CAs damages induced by RT. The aim of the present study was to determine the incidence of CAs and AMN in peripheral lymphocytes of patients with lung cancer exposed to γ radiation due to treatment.

Materials and Methods

Chemicals: RPMI 1640 medium with L–glutamine (cat.01-100-1), foetal bovine serum (heat inactivated, cat.04-121-1), colcemid solution (concentration: 10 μg ml⁻¹, cat.12-004-1), phytoheamagglutinin (cat.01-198-1) and penicillin-streptomycin solution (cat.03-031-1) were obtained from Biological Industries Ltd, ISRAEL.

Patients and Treatment: The present study has been carried out on 20 patients treated for lung cancer from November 2004 to July 2006 in Ankara University Andicen Polyclinic of Dr. Abdurrahman Yurtaslan Research Hospital. The patients were selected randomly. Of these patients, seventeen were males and three were females. The mean age of the patients was 53.5 ± 2.8 yrs (range 45–60). Small cell lung cancer was diagnosed in 11 (55%) patients, adenocarcinoma – in 4 (20%), large cell lung
cancer – in 3 (15%) and squamous cell lung cancer – in 2 (10%) patients. Stage I cancer was diagnosed in 2 (10%), stage II – in 5 (25%), and stage III and IV – in 13 (65%) patients. Histologically, small cell lung cancer was predominant (11 cases). All patients had smoked more than 20 cigarettes per day for at least 20 yr(s). Besides, sixteen patients were current cigarette smokers. All of the patients had respiratory dysfunction. Lung cancers were located in the upper, lower and middle lobes of lung. Patients were not to have taken any chemotherapeutic drugs during radiation period. Table 1 shows the features of subjects used in this study.

**Ethical standards:** In this study, the methods and techniques applied to patients carried out favorably to ethical standards of local ethical committee of Abdurrahman Yurtarslan Research Hospital (Protocol date: 27.10.2005) and favorable to the guidelines set by the World Health Organisation (Geneva, Switzerland). Each patient signed an informed–consent form before participating in the study. This permission is always for the analysis and collection of blood from patients, and it are not used for purposes other than those for which consent was originally given.

**Radiation treatment:** Radiation procedure was carried out using “ATC Cobalt 60 SSD=80 cm” instrument. The radiation doses were calculated by requirements of lung cancer treatment that recommended by International Commission for Radiation Protection (ICRP). Totally 20 patients were treated with 10 Gy γ-radiation during five wk(s). They received γ-radiation during five wk(s) at 10 Gy dose per wk in 50 Gy total dose. The radiation was applied on thorax area during 30 min. Cytogenetical analyses were performed on lymphocytes of patients exposed to radiation.

**Lymphocyte sampling and culture:** Peripheral blood was sampled from 20 patients with lung cancer and collected before and after RT. Three milliliters (3.0 ml) of venous blood was obtained from each patient, and evacuated into heparinized tubes, and transported to the laboratory on the same day. In the laboratory culture was prepared according to Scarfi et al. (1994) and Siddique et al. (2007). Briefly, 1.0 ml of whole blood was immediately added to 10 ml of RPMI 1640 medium containing 10% heat inactivated fetal calf serum, 0.3 ml phytohaemaglutinin, 150 U/mL penicillin and 150 mg/l streptomycin. Then cell culture was incubated at 37°C for 72 hr in a humidified atmosphere containing 5% CO₂ in air.

**Metaphase preparations:** At the end of 70 hr incubation, colcemide, was added to block cells in metaphase during the last 2 hr of cell growth. Then standard cytogenetics procedures as hypotonic treatment, fixation, slide preparation and staining was performed (Movafagh et al., 2007). The metaphase preparations are made by placing 10-20 μl of cell suspension with a pipette on freezing slides.

**Analysis of cells:** Aberrant metaphase number (AMN) was counted as the number of damaged metaphases among 75 metaphases in each culture.

**Chromosomal aberration analysis:** From each patient 75 metaphases were counted for CAs such as break, dicentric, ring, gap and their were considered to be equal. CAs were scored with binocular light microscope (Japan, Nicon Eclipse E600) according to scoring criteria of Testard et al. (1996), Buckton and Evans (1973), Kilbey et al. (1997), Siddique and Afzal (2005).

**Statistical analysis:** We calculated the 95% confidence limits according to Standard determinations for the data of CAs and cumulative dose-effect curves. For each data Paired Samples t-test was applied to compare the mean values of abnormalities.

**Results and Discussion**

**The frequency of CAs:** When peripheral lymphocytes are exposed to radiation, all aberrations occurred in metaphases in the first cell division after radiation exposure with RT treatment (Hayata, 2000). Surprisingly, we did not observe any CAs in lymphocytes before RT in the present study (Fig. 1A, Table 2). Whereas, recently studies on human populations have indicated a positive relation the frequencies of CAs in peripheral lymphocytes and cancer (Natarajan, 2002). The most of the damaged cells showed a large number of CAs such as chromosome breaks, dicentrics, rings and chromosome gaps (Fig. 1B-D, Table 2). The highest CAs frequency was observed at radiation treatment at the end of fifth wk. The least frequency of CAs was observed at 10 Gy dose at the end of first wk. These results show that the frequency of CAs were cumulative dose dependent. Moreover, number of chromosome breaks were

**Table - 1:** Categorization of subjects

<table>
<thead>
<tr>
<th>Lung cancer types</th>
<th>Male patients</th>
<th>Female patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small cell lung cancer</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Large cell lung cancer</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Squamous cell lung cancer</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

**Table - 2:** The frequency of CAs and AMN in lymphocytes exposed to γ-radiations during radiotherapy

<table>
<thead>
<tr>
<th>Radiation treatment</th>
<th>Break</th>
<th>Dicentric</th>
<th>Ring</th>
<th>Gap</th>
<th>AMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before RT</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>First wk</td>
<td>10.15±2.23</td>
<td>4.36±1.13</td>
<td>1.60±0.94</td>
<td>0.40±0.50</td>
<td>9.30±1.84</td>
</tr>
<tr>
<td>Second wk</td>
<td>13.55±2.24</td>
<td>6.15±1.39</td>
<td>2.60±1.10</td>
<td>0.90±0.64</td>
<td>13.80±2.09</td>
</tr>
<tr>
<td>Third wk</td>
<td>16.40±5.52</td>
<td>7.95±1.32</td>
<td>4.35±1.14</td>
<td>1.45±0.76</td>
<td>20.40±2.04</td>
</tr>
<tr>
<td>Fourth wk</td>
<td>20.35±2.35</td>
<td>10.25±1.55</td>
<td>5.60±1.35</td>
<td>2.30±0.66</td>
<td>29.35±3.01</td>
</tr>
<tr>
<td>Fifth wk</td>
<td>24.70±1.81</td>
<td>12.50±1.28</td>
<td>7.05±1.10</td>
<td>3.20±0.77</td>
<td>36.15±1.81</td>
</tr>
</tbody>
</table>

*Values presented as mean±SD (n=20)
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higher than other CAs (as dicentric, ring and chromosome gap), and difference was statistically significant (p<0.05). The incidence of CAs was found break>dicentric>ring>gap (Fig. 2). All these abnormalities showed an increase from 186 to 723 from first treatment to fifth treatment, respectively. Atricentric and acentric chromosome were also observed in Fig. 1B and 1C, demonstrating the existence of complex re-arrangements. Complex and simple CAs are a characteristic feature of radiation and may be a potential biomarker of exposure (Ballarani and Ottolenghi, 2003).

The level of abnormal metaphases: In Fig. 3, the level of abnormal metaphases induced by RT are evaluated for cumulative dose values in the first and fifth wk(s) range in 20 patients. The total metaphases abnormalities were increased with cumulative radiation dose. The results described above are significantly different at p<0.05 when compared to the before RT. These results were fitted to the dose-effect model as mentioned above. 12.4, 16.6, 27, 38.4 and 43.5% of total metaphases were scored as abnormal metaphases in the treatment of first-fifth week(s), respectively.

Several studies have been performed on the induction of CAs in human lymphocytes by radiation. Legal et al. (2002) reported that increased of CAs frequency after RT and chemotherapy in lymphocytes of patients with breast carcinoma. In a similar study, CAs in human sperm and lymphocytes were compared before and after in vivo radiation treatment of 13 cancer patients. As a result, it was demonstrated that there were no abnormalities in sperm or lymphocytes before RT. But, following RT there was an increase in the frequency of numerical and structural chromosomal abnormalities in both lymphocytes and sperm (Martin et al., 1989). In another study, it was investigated frequency of stable chromosomal aberrations (SCAs) in circulating lymphocytes of patients with breast carcinoma exposed to RT alone (n=15) or RT combined with chemotherapy (n=10). As a result, it was demonstrated that in all patients, the rate of SCAs increased significantly after external irradiation (Legal et al., 2002). Movafagh et al. (2007) investigated the frequency of CAs in lymphocytes of RT workers after first mitotic division in Tehran, Iran. At result, they showed that increased of CAs such as dicentrics, acentrics and ring chromosomes in the experimental group.

Our results are in accordance almost with the previous studies available so far and mentioned above. In fact, in most studies radiation has been shown similar effects. However, this is the first study that indicative not observed of CAs before RT in lymphocytes of patients with lung cancer. Consequently, exposure to γ-radiation during RT increases the frequency of CAs, and this condition is a significant the risk for health. These damages may be developed secondary diseases such as leukemia and anaemia (Maffei et al., 2002; Muirhead et al., 1999). Therefore, effects of RT applications...
on healthy cells must be minimized or alternative methods should be developed.

References