A Preliminary Study on DNA Damage in Peripheral Blood of Cancer Patients After Radiotherapy with Comet Assay

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Abstract The aim of this study was to look at DNA damage studied in cancer patients during radiotherapy using the comet test. Subjects were from patients diagnosed with different solid tumors who received radiotherapy and control groups adjusted for age, sex. Blood samples were collected from patients with several types of cancer after receiving cumulative radiation doses of 10, 12 and 14 Gy. The level of DNA damage was evaluated using the alkaline comet assay on peripheral blood lymphocytes. Samples consisting of 3 ml of whole blood were collected by venipuncture. Isolation of lymphocytes was done using gradient centrifugation method. Evaluation on the capability of this compound in suppressing DNA damage was done by using alkaline Comet assay and The frequency of DNA cells was evaluated by calculating a total of 50 cells per slide. Data analysis was done using CaspLab program. The comet test results showed that the average tail length, tail DNA, and tail moments of radiotherapy patients were higher than the control group and significantly different between both group (P <0.01). There is a positive correlation between radiation doses received by patient with DNA damage. The conclusion is that exposure to ionizing radiation leads to an increase in the level of DNA damage in the patient's peripheral blood lymphocytes. Meanwhile, it was found that there was inter-individual variability in response to radiotherapy among patients.

Keywords: DNA damage, cancer, radiotherapy, comet assays

I. INTRODUCTION

Cancer is a degenerative disease characterized by uncontrolled cell division (proliferation) and can cause the ability of cells to migrate to other body tissues through blood circulation or the lymphatic system (metastasis) in pathology. Cancer is a major health problem worldwide and the second biggest killer disease after cardiovascular disease. In developing countries cancer is one disease with very many sufferers. Radiation therapy is an efficient treatment for cancer [1].

Radiotherapy is a treatment aimed at shrinking mass tumors or removing residual tumor cells by exposing the tumor to ionizing radiation. Radiotherapy mostly uses X and gamma ray radiation [1,2]. Radiation besides affecting tumors also affects healthy cells around it. Ionizing radiation causes DNA damage in both direct and indirect effects. Radiation causes the formation of ROS (reactive oxygen species) which is indirectly involved in DNA damage (3,4). Radiosensitivity is the susceptibility of cells or tissues to ionizing radiation. Some patients may be more sensitive to radiation. Sensitivity results from the toxic effects of radiotherapy which results in lesions in normal tissue patients (5).

The latest literature in the field of clinical oncology and radiotherapy still does not adequately explain the ratio of damage to non-tumor cells and tissues after radiation treatment. Ionizing radiation is a proven mutagen, besides damaging tumor tissue, it also causes damage to genomes in other cell (3,4,6). Peripheral blood lymphocytes are most often monitored to see genome damage caused by radiation or other mutagens. It is important to monitor lymphocyte cells in radiotherapy patients to studying the DNA repair process in various cytogenetic biomarkers. This can indicate the individual's sensitivity to radiotherapy and the potential risk for the appearance of secondary tumors (7). Thus, cytogenetic and molecular-biological tests need to be carried out in oncological patients. This is to monitor the increase in the frequency of chromosomal aberrations and the increase in the number of micronucleus and the level of DNA damage in peripheral blood lymphocytes in oncological patients treated with radiation (6).

Comet Test is a simple, fast and sensitive visual method for measuring and analyzing DNA damage Single strand break and Double strand break. This technique is carried out at the individual cell level and requires only a small number of cells in each sample. The comet test is an electrophoresis method that estimates damage by measuring the migration of DNA fragments (8). Comet alkaline tests are reported to detect DNA damage at radiation dose limits of up to 0.6 cGy. This method provides important information about the risk of diseases associated with oxidative stress. Over the
The Comet Assay software (13).

III. RESULTS

This research is a preliminary study to look at DNA damage from cancer patients after radiotherapy. Blood samples from patients with various types of cancer with different therapeutic doses. Individual data from sufferers of several types of cancer Individual data on DNA damage recorded in peripheral blood leukocytes are reported in Table 1.

TABLE 1. ANAMNESTIC AND CLINICAL DATA ON CANCER PATIENTS INVOLVED IN THE STUDY

<table>
<thead>
<tr>
<th>No</th>
<th>Age (Year)</th>
<th>Gender</th>
<th>Cancer</th>
<th>Total Doses (Gy)</th>
<th>DNA migration/ Tail Length (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSJ4</td>
<td>29</td>
<td>L</td>
<td>Sarcoma extemity</td>
<td>10</td>
<td>21.74</td>
</tr>
<tr>
<td>PSJ5</td>
<td>51</td>
<td>L</td>
<td>Submandibula Sinister</td>
<td>14</td>
<td>31.32</td>
</tr>
<tr>
<td>PSJ7</td>
<td>21</td>
<td>L</td>
<td>Parotis</td>
<td>12</td>
<td>26.24</td>
</tr>
<tr>
<td>PSJ11</td>
<td>48</td>
<td>P</td>
<td>Breast</td>
<td>12</td>
<td>31.1</td>
</tr>
<tr>
<td>PSJ12</td>
<td>61</td>
<td>L</td>
<td>Nasopharynx</td>
<td>12</td>
<td>26.4</td>
</tr>
<tr>
<td>PSJ5</td>
<td>68</td>
<td>P</td>
<td>Tongue</td>
<td>14</td>
<td>34.16</td>
</tr>
<tr>
<td>PSJ6</td>
<td>61</td>
<td>P</td>
<td>Parotis</td>
<td>12</td>
<td>29.86</td>
</tr>
<tr>
<td>PSJ7</td>
<td>45</td>
<td>P</td>
<td>Breast</td>
<td>14</td>
<td>31.06</td>
</tr>
<tr>
<td>PSJ22</td>
<td>57</td>
<td>P</td>
<td>Humeri+Anterabrach</td>
<td>10</td>
<td>30.02</td>
</tr>
<tr>
<td>PSJ24</td>
<td>48</td>
<td>P</td>
<td>Tongue</td>
<td>10</td>
<td>31.06</td>
</tr>
</tbody>
</table>

DNA damage in patients after radiation will be compared with DNA from control samples.
After the comet assay is carried out then an observation is carried out under the fluorescent microscope. In this research, the DNA damage of irradiated lymphocytes was assessed by comet assay by staining the cells with ethidium bromide and the comet that mainly consist of single stranded DNA can be seen with a fluorescence microscope as presented in Figure 1.

![A and B](image)

**FIGURE 1. VISUALIZATION OF THE RESULTS COMET ASSAYS OF DNA LYMPHOCYTE OF CONTROL (A) AND PATIENT (B).**

Results of visualization showed that lymphocyte cells patient formed tail of comet than control due to DNA damage in the form of breaking one DNA strand (single strand break/SSB) and the rupture of both strands of DNA at the opposite position (double strand breaks/DSB) (Fig. 1). Denatured DNA fragments that migrate out of the cell nucleus during the electrophoresis process. The migration will form a comet’s tail while the non-deposition area will form a comet head. (6,11). The DNA damage can be estimated by measuring the length of the comet tail using image analysis software.

![Graph](image)

**FIGURE 2. DNA MIGRATION (TAIL LENGTH) OF THE COMET ASSAY RESULTS ON CONTROL AND PATIENT SAMPLE.**

The results of calculation with the Casplab software that’s of the length of the comet tail (DNA migration) is controls 8.02 - 37.86 with an average value of 16.51 ± 8.81. DNA migration of patients ranged from 21.74 - 34.16 with an average of 29.2 ± 3.5. There was a significant difference between the two groups at p <0.05.(Figure 2)

In this study based on the type of organ in the cancer are grouped into 3 namely, Breast, Head and neck (Submandibules Sinister, Nasopharynx, Parotid, Tongue), and extremity (Sacrom extremity, Humeri+Anterabracchi). The results of calculation with the Casplab software that’s of range and average the length of the comet tail (DNA migration) as follow, the breast cancer is 31.06 - 31 and 31.08±0.03 , Head & neck is 25.4 - 34.16 and 29.67±3.3 , extremity is 21.74 - 30.02 and 25.88±5.8 (Figure 3).
The fractionation dose range that’s received by patients is 10–14 Gy. The effect of doses on DNA damage is shown on the correlation curve between doses with a tail length value (Figure 4). The curve shows a positive correlation between the doses and DNA damage with a value of $R^2 = 0.2623$.

The curve relations curve between doses with a tail length value shows a positive correlation. This shows that the greater the radiation dose received by the patient will result in increased DNA damage in the patient.

IV. DISCUSSION

As observed in our study, radiotherapy critically affected the level of DNA damage in cancer patients, as detected by the alkali comet test. In the results of the study above the DNA damage patients was significantly higher than the control. This shows DNA damage in patients receiving radiotherapy. The same study was also carried out by Gamulin et al. in 10 patients with different types of cancer. Showed a significant increase in TL values in 6 patients after radiotherapy (14). Higher radiation received the greater the DNA damage that occurs. DNA damage in patients receiving the same dose shows varying DNA (TL) migration values. This shows that there is an inter-individual variation of course related to age and some lifestyle factors (especially smoking habits and alcohol consumption), as well as previous medical measures, correlated to inherited biological factors.

Radiotherapy is a major part of the treatment of cancer patients. This can be used as the main therapy, but often combined with surgery and chemotherapy. Radiotherapy works by damaging the cancer cells of DNA, therapeutic interventions cause inevitable exposure of non-target cells to patients (3,14,15). Because only a portion of the population treated will develop secondary cancer so that the patient's biomonitoring after therapy becomes very important (1,9,16).

V. CONCLUSION.

Radiotherapy is accompanied by significant DNA damage in peripheral blood lymphocytes. DNA damage has a positive correlation with the dose received by the patient.

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