







Evaluation of Genotoxicity Risk in Health Care Workers Exposed to Antineoplastic Drugs

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ABSTRACT

Objective: DNA damage that can be caused by workplace exposure to antineoplastic drugs in health workers has been shown in many scientific studies. It is aimed to evaluate whether the risk of genotoxicity in health workers decreases after the regulations and measures taken by national and international health authorities in our work.

Methods: For this purpose, DNA damage was assessed by using alkaline comet technique in lymphocytes isolated from blood samples of health workers (n=29) who were involved in preparing and / or administering antineoplastic agent at Trakya University Health Research and Application Center and compared with the control group (n=30). Also, those who prepare and/or administer antineoplastic agents; (n=16) and manual (n=13) preparations.

Results: As a result of the evaluation, there was no statistically significant difference between health personnel and control group in preparing and / or administering antineoplastic agent ($p>0,05$, Mann-Whitney U) and there was no difference in the genotoxic risk between preparation forms. Furthermore, when the exposed control group was assessed for DNA damage as smokers and nonsmokers, there was no statistically significant difference in terms of DNA damage ($p>0.05$).

Conclusion: At the center where our samples were taken, the resulting measures resulted in the control of the risk of genotoxicity due to occupational exposure to antineoplastic agents.

Keywords: Alkaline Comet Assay, Genotoxicity, Antineoplastic, Occupational exposure, Occupational health safety

1. INTRODUCTION

Antineoplastic agents are drugs that are used in the treatment of cancer and have mutagenic and carcinogenic properties which affect healthy cells because of their low selectivity to cancerous cells. Health workers are exposed to contaminants such as tears, saliva, sweat, and contact with body wastes such as urine, feces, vomit, etc. during preparation and administration of these drugs during the cleaning of dusts and spillages caused by breakage of tablets (1).

Studies have shown that workplace exposure to antineoplastic medicines causes DNA damage in health workers (1). This poses a risk for the fetus if it is risky for health workers and if the health worker is unaware of the fact that she is pregnant (2). It is important to note that the duration of exposure and the precautions specified in the safe use standards of antineoplastic medicines published by the Ministry of Health (such as the use of gloves and goggles, preparation in biological safety cabin) are significant during this risk (3).

Since they are mutagenic and carcinogenic, a dose that can be considered safe for exposure to these drugs can not be determined. The assessment of genotoxicity risk is very important in terms of protection of the health of the health

care workers, because this exposure is reduced as much as possible and at low doses, because of the possibility of continuous exposure to these drugs. Recently, robotic drug preparation units have been used to reduce occupational exposure and minimize errors in drug preparation. The aim of our study is to evaluate the current status of the genotoxicity risk reported in previous studies in the health care workers working in the preparation unit of antineoplastics such as doxorubicin, 5-fluorouracil, docetaxel, paclitaxel and cyclophosphamide by using alkaline comet technique.

2. METHODS

2.1. Chemicals and Reagents

The chemicals used were the following: disodium ethylenediaminetetraacetic acid (Merck 324503), low melting agarose (LMA) (Sigma A4018), high melting agarose (HMA) (Sigma A7174), sodium hydroxide (Sigma 06203), sodium chloride (Merck 106404), Tris (Sigma T6066), Histopaque 1077 (Sigma 10771), Ethidium Bromide (Sigma E8751), Hydrochloric Acid (Sigma 320331), Triton X-100 (Fisher BioReagents bp151-100), ethanol (Merck 100983)

2.2. Collection of Working Group and Blood Samples

Between the years 2015-2017; health workers (Exposed group, $n = 29$) taking part in preparing and/or administering antineoplastic agents such as doxorubicin, 5-fluorouracil, paclitaxel and cyclophosphamide were included in Health Research and Application Center of Trakya University for at least 3 months and health workers (Exposed group, $n=30$) were compared in the same hospital with no antineoplastic agent and with similar demographic characteristics (age, gender, alcohol, smoking, etc.). Also, those exposed to antineoplastic agents are evaluated as robotic ($n = 16$) and manual ($n = 13$) preparations. The suitability of the study for the Helsinki declaration was approved by the Scientific Research Ethics Committee of the Faculty of Medicine of Trakya University (Decision No: TÜTF-GOKAEK 2014/107). Individuals were informed about the study first, and 2 consecutive venous blood samples were collected in heparinized tubes after the consent form and questionnaire were filled out voluntarily from those who agreed to participate in the study. Pregnants and those who received x-ray radiation in the last 6 months and those who did heavy workouts in the previous 3 days were not included in the study.

2.3. Lymphocyte isolation

Blood, which was brought to the laboratory rapidly after it was received, was centrifuged with histopaque 1077 to isolate lymphocytes (250g, + 4°C, 10').

2.4. Alkali Comet Technique

Alkali comet technique developed by Singh et al and adapted to our laboratory was used to determine DNA damage in isolated lymphocyte samples (4). The slides were covered with 0.65% high boiling grade agar (HMA) 1 day prior to the experiment. Stock lysis solution (2.5M NaCl, 100 mM Na₂EDTA, 10 mM Tris, pH10), 10M NaOH solution, 0.2 M EDTA solution and neutralization buffer (0.4 Molar Tris, pH 7.5) were prepared overnight and stored at + 4°C.

Two specimens were used for each sample. 100 µL of the isolated lymphocyte suspension and 0.65% low boiling-point agar (LMA) (37 ° C) were spread on the slide and covered with lamellae. The slides were left in the cold for 30 minute to solidify the agar and cell suspension and then lysed overnight at + 4°C in a freshly prepared lysis solution (stock lysis solution, Triton X-100 and DMSO; 89%: 1% 10%) to lyse lymphocyte cells.

2.5. Electrophoresis and dyeing

The laminates removed from the lysis buffer were left in the electrophoresis solution [300 mM NaOH, 1 mM EDTA pH13] for 20 min to open the DNA helix. Subsequently, the electrophoresis was placed in a horizontal electrophoresis tank and subjected to electrophoresis for 20 minutes at 15 volts at 300 mA. All post-lysis procedures were performed in the dark and at + 4°C to avoid additional DNA damage. The

slides removed from the electrophoresis were neutralized in neutralization buffer [0.4 Molar Tris, pH 7.5] 3 times for 5 minute. Lastly, 50%, 75%, 100% alcohol was held for 5 minute at + 4°C, and the slides were fixed and dried. Each slide was examined by staining with 50 µL Ethidium Bromide (EtBr – 20 µL/mL).

2.6. Microscopic Analysis

The fluorescence attenuated microscope (Axio Observer Z1, Carl Zeiss, Germany) scored comet in 100 cells per well with 40x magnification. Scoring; according to the traction of the comet tail in the electric field, it was classified into 3 groups as non-immigrant, less immigrant, and immigrant. Total comet score is calculated with 0x (non-migrated comet) + 1x (few comet) + 2x (comet with high migration) formula (5). For each slide, the damage was scored from 0 (no damage) to 200 (maximum damage).

2.7. Statistical analysis

It was calculated that when trying to find a significant difference of 0.04 in the groups, 99% of the effort and 0.01 of the α error level were found, 28 participants were required in each group. Groups were followed by 29 control groups and 30 study groups. Statistics were expressed as descriptive variables, mean and \pm standard deviation, median, percentile. Categorical variables were compared with chi-square and fisher tests. Shapiro-Wilk's test is used to assess normality of the variables. Student's T test is used for variables that follow normal distribution and Mann-Whitney U test is used for variables that does not follow normal distribution. A value of $p < 0.05$ was considered statistically significant.

3. RESULTS

As shown in Table 1, there was no statistically significant difference between the distribution of demographics and life habits in the study and control groups ($p > 0.05$). There was no statistically significant difference in total Comet Score that was a DNA damage parameter in peripheral blood lymphocytes, when compared between health personnel and control group taking part in preparing and/or administering antineoplastic agent (Table 2, $p > 0.05$). The exposed group was divided into two subgroups in order to assess the effect of the preparation on the risk of genotoxicity. First manual subgroup is formed from workers who prepare the drug manually; and/or health-care workers who apply the manually prepared medication to the patient. The second is the robotic subgroup; a robotic unit, and a healthcare worker who prepares a medicine or applies a medicine to a patient who is prepared in a robotics unit. There was no statistically significant difference between Total Comet Score manual subgroup and robotic subgroup (Table 2, $p > 0,05$).

Furthermore, when the effect of DNA damage was assessed in smokers in the groups, no statistically significant DNA

damage results were found with non-smokers (Table 2, $p > 0.05$).

Table 1. Demographic characteristics and lifestyle habits of the groups

| Groups | | Control group | Exposed group | p value | |
|-----------------------------|------------|--------------------|------------------|-----------|--------|
| n | | 30 | 29 | | |
| Age mean (\pm SS) (year) | | 34.67 \pm 8.65 | 32.28 \pm 7.41 | 0.260* | |
| Body mass index (\pm SS) | | 24.7707 \pm 4.69 | 23.43 \pm 3.39 | 0.213* | |
| Gender | Woman | 26 (86.7%) | 24 (82.8%) | 0.731* | |
| | Man | 4 (13.3%) | 5 (17.2%) | | |
| Smoking | Smoker | 1-10 (piece/day) | 4 (13.3%) | 5 (17.2%) | 0.306* |
| | | 11-20 | 8 (26.7%) | 4 (13.8%) | |
| | | \geq 21 | 2 (6.7%) | 0 (0%) | |
| | Non-smoker | 16 (53.3%) | 19 (68.4%) | | |
| Alcohol consumption | Yes | 9 (30%) | 4 (13.8%) | 0.209* | |
| | No | 21 (70%) | 24 (82.8%) | | |
| | Quitter | 0 (0%) | 1 (3.4%) | | |

+: Student's T test; *: Fisher's exact test; exposed group against control group

Table 2. DNA damage (TCS)* according to control and study groups and preparation patterns

| Groups | | n | Median (25 th percentiles – 75 th percentiles) | $p^{\$}$ |
|---------------|-------------------------------|----|--|----------|
| Control Group | | 30 | 0.00 (0.00 – 2.25) | 0.140* |
| Exposed group | | 29 | 2.00 (0.00 – 3.00) | |
| Control Group | Smoker | 14 | 0.5 (0.00 – 3.25) | 0.322* |
| | Non smoker | 16 | 0.00 (0.00 – 1.75) | |
| Exposed group | Smoker | 9 | 1.00 (0.00 – 2.00) | 0.302* |
| | Non Smoker | 20 | 2.50 (0.00 – 5.25) | |
| Exposed group | Manuel Preparation | 13 | 3.00 (0.00 – 6.00) | 0.337# |
| | Preparation with Robotic Unit | 16 | 1.00 (0.00 – 2.75) | |

(\$: Mann-Whitney U test is used; +: working group against control group; #: Preparation with robotic unit subgroup against preparation manual subgroup; *: TCS (Total Comet Score) = 0x (number of non-migration comet) + 1x (few comet) + 2x (high migration number comet)

4. DISCUSSION

The deterioration of working health after occupational exposure is a major problem both in terms of public health and the health economy. Today, health and work authorities take various measures and apply sanctions for occupational health and safety. In the last 30 years, attention has been drawn to the possible exposure risk of health personnel preparing and administering antineoplastic drugs. In 2004, T.C Ministry of Health General Directorate of Treatment Services issued "Guidelines for Safe Work with Antineoplastic (Cytotoxic) Drugs" to inform relevant health personnel and provide a safe working environment (3). In our study, genotoxicity risk was assessed by alkaline comet technique after taking precautions in health personnel involved in preparing antineoplastics such as doxorubicin, 5-fluorouracil,

mossataxel, paclitaxel and cyclophosphamide, and genotoxicity was not detected in control subjects exposed to antineoplastic agents. Furthermore, the preparation did not have a statistically significant role on DNA damage in the group included in the study.

The risk of genotoxicity caused by exposure during the administration and preparation of medicines in health workers has been evaluated in various studies, and it has been shown that some studies have no statistically significant risk of genotoxicity (6,7) and some studies have a statistically significant effect on DNA damage compared to the control group (5,8-17). One of the first studies done in nurses exposed to antineoplastic drugs in Turkey has started in 1991 Sardas et al. In this study, it was reported that sister chromatid technique detected high chromatin damage in lymphocytes compared to the control group (8). Some examples of DNA and chromosomal damage reported by different techniques are presented below. El-Ebiary et al. reported higher chromosomal aberrations and microcirculation frequency in the study of chromosomal aberrations and microcirculation in healthcare personnel in preparing and administering antineoplastic drugs in a cancer hospital (1). Burgaz et al. reported a higher microcirculation rate in healthcare personnel in our country than in the control group by means of microcephaly method in our country where they detected antineoplastic drugs in urine specimens (9). In a similar study, it was reported that genetic damage was not observed with comet technique in health personnel who had antineoplastic drugs in urine specimens in America (6). In two different studies, Villiarini (10) and Rekhadevi (11) et al. reported that DNA damage was found to be statistically significantly higher in healthcare personnel who prepared an antineoplastic drug in studies evaluating exposure to urine, and that the damage was less with Villiarini and arc protective equipment. In another study that showed that protective equipment reduced the risk, Kopjar anda et al. also reported high levels of damage in the health care staff who prepared antineoplastic medication compared to the control (12). Maluf et al. reported that there was no difference in micro-nuclear parameters after a new evaluation, which was the continuation of the study after 4 years in the group they had previously performed and those who were exposed to antineoplastic agents in the work environment, found that the frequency of DNA damage was significantly higher than that of control (13).

When the results of the studies are examined, it is seen that individual factors such as age, drugs used, life style, smoking and alcohol use, it is seen that method-dependent variables such as exposure time, dose, application frequency and combination, accidental drug delivery, biological safety cabinets, glove, glasses and mask dependent factors such as the time taken for blood sampling, method differences in the applied comet technique, (6,10,18,19).

It is known that different antineoplastic agents exhibit different DNA damage profiles and produce synergistic effects together (20). Limitation of our work is study results cannot

be generalized, because of the differences between health care workers such as exposure time, application frequency and safety precautions. The alkaline comet technique, which we use in our study, is a technique that is frequently used in human biosimulation studies and accepted as correct (21,22).

In the first study conducted in 1998 with the alkaline comet technique in our country for the evaluation of genotoxicity in nurses who prepared antineoplastic medicines, Undeger et al emphasized that the genotoxicity risk observed in our country was due to the lack of guidelines to provide awareness of health personnel in our country (14). In another study conducted in our country, İzdes et al reported the risk of DNA damage in nurses exposed to antineoplastics (5). In our study, in the health personnel included in the study, the results were not statistically significant but the total comet score average was found to be lower when working with the robotic unit. In the robotic drug unit, the medicines are prepared automatically and the prepared health care practitioner applies to the patient. The risk of exposure to the robotic unit is therefore reduced. In the study conducted by Sessink et al., low level surface contamination was detected in some vials due to spillage in the robotic drug preparation unit, but due to the wearing of two layers of gloves, it was shown that no contamination of workers' hands and no cyclophosphamide in their urine (23).

5. CONCLUSION

As a result, it has also been observed in our work that the use of protective equipment such as gloves, masks, goggles, cabin, and the necessity of working with a robotic drug unit (10,14,17,24). As emphasized in other studies (1) occupational exposure determination and risk assessment studies are specific to the population in which they are conducted and are not realistic to compare with each other.

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