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Original article

The effect of X-ray irradiation to the formation of polychromatic erythrocyte cell micronucleus in Wistar rats (*Rattus norvegicus*)

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ABSTRACT

Background: *Panoramic and cephalometric radiography is very important for diagnosis, treatment plan, and evaluation of orthodontic treatment results. Panoramic and cephalometric radiography are frequently performed at the same time, causing DNA damage and chromosome aberration.* ***Purpose:*** *This study aims to analyse the effect of X-ray exposure in panoramic and cephalometric radiography on micronuclei cell numbers.* ***Methods:*** *Laboratory-based analytical study with 60 healthy-male Wistar rats weighing 200–300 grams divided into 6 treatment groups (n=10). The control group: without radiographic exposure, the treatment group 2: using panoramic radiographic exposure followed by cephalometric, and the treatment group 3: using panoramic radiographic exposure and 24 hours later performed cephalometric radiographic. The unit of analysis was the polychromatic erythrocytes of mice cell, were examined 24 hours and 48 hours after irradiation had been finished. The polychromatic erythrocytes were examined using May-Gruenwald-Giemsa staining and 100x magnification under a microscope with 2000 cells per view. Data obtained were analysed using the Statistical Package for Social Science (SPSS) 20 version software. The mean and standard deviations were calculated for each clinical parameter, and a one-way ANOVA statistical test of significance was used. Statistical significance was set at $p<0.05$.* ***Results:*** *The analysis showed a significant increase ($p<0.05$) in the number of micronucleus in groups that used panoramic radiographic exposure followed by cephalometric.* ***Conclusion:*** *X-ray radiation can increase the number of micronucleus in polychromatic erythrocyte cells in rats.*

Keywords: *cephalometric X-ray; micronucleus; panoramic X-ray; polychromatic erythrocyte cell*

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INTRODUCTION

Radiographic examination denotes essential steps in dentistry, especially in clinical orthodontics.[1] Panoramic imaging and cephalometric analysis are required before treatment to help make an established diagnosis and consider several treatment options.[2] The processes of panoramic and cephalometric radiography are mostly done in succession to save the patients time and energy.[3] Besides, taking panoramic and cephalometric imaging at the same time also hasten diagnosis establishment and treatment planning process. However, this decision gives rise to several undesirable effects toward cells in the body such as the DNA single-strand break, DNA double-strand break, DNA cross-link, and chromosome aberrations.[4]

Chromosome aberration is an immediate effect of radiation exposure. Therefore, radiation protection is needed for patients and operators.[5] Chromosome aberrations can affect one or more genes in a complex manner by changing the regulation of gene expression, disrupting exons, and creating fusion genes.[5] The examination of chromosome aberration can be done using a micronuclei test. In the telophase stage, the chromosome fragment and chromatin inside the cell will fall behind in the cytoplasm forming a nucleus-like structure, with sizes ranging from 1/20 to 1/5 of the nuclei diameter, called

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micronucleus.[6] The micronucleus formation denotes an indicator of mutagenic activity that may damage the chromosome, leading to cancer.[7] A simple method to detect micronuclei can be done by examining polychromatic erythrocyte cells from the mice bone marrow swab.[8] This study aims to analyse the effect of X-ray exposure in panoramic and cephalometric radiography on micronuclei cell numbers.

MATERIALS AND METHODS

All the procedures in this study have been reviewed and approved by the Faculty of Dental Medicine, Universitas Airlangga Ethical Committee, with ethical certificate number 395/HRECC.FODM/VI/2019. This was a laboratory-based analytical experiment involving 60 male Wistar rats, three to four months old, weighing 200–300 g. The sampling technique from the rat's right femur was determined by Lemeshow's method. The rats were acclimatized for a week and randomly divided into six groups, each containing ten samples, namely A1, B1, A2, B2, A3, and B3. A1 and B1 act as the control groups receiving no radiation; while A2 and B2 groups were exposed to X-ray radiation from panoramic and cephalometric radiographs consecutively. The A3 and B3 groups were exposed to X-ray radiation from a panoramic radiograph and were re-exposed to X-ray radiation from a cephalometric radiograph after 24 hours. The observation of micronuclei for groups A1, A2, and A3 was conducted 24 hours after radiation exposure while the observation for groups B1, B2, and B3 was done 48 hours after radiation exposure.

The rats were kept in a plastic cage at room temperature with a 12-hour light-dark cycle at a constant temperature of 23°C and fed a standard pellet diet (expanded pellets, Stepfield, Witham, Essex, UK) with tap water *ad libitum*. The X-ray exposure was done according to the group allocation with doses of 0.3 mSV for panoramic radiograph and 0.03 mSV for cephalometric radiograph using the X-ray machine Orthopantomograph® OP100 (Instrumentarium Corporation, U.S) with a capacity of 77 kVp 12 mA. After being exposed to the X-ray radiation, the rats were put back in the cage. Twenty-four hours after the X-ray exposure, rats in groups A1, A2, and A3 were euthanised by means of cervical dislocation while the rats in groups B1, B2, and B3 were euthanised 48 hours after, in the same manner. Bone marrow was aspirated with a 5-ml syringe slowly from the femur, moved to a micro-tube, and centrifuged consecutively. After a suspension was formed, a drop of the suspension was put on an object-glass, dried, and stained using May-Gruenwald-Giemsa. The bone marrow slides were observed after oil immersion under a light microscope (BX 53 upright microscope, Olympus Corporation, Tokyo, Japan) at 100x magnification. The micronuclei in polychromatic erythrocyte counting were conducted using the score blind method by means of a cell counter. In this study, micronuclei from 2000 polychromatic erythrocytes were counted by three researchers after calibration between the researchers. The data obtained were analysed using SPSS version 20 for Windows (IBM Corp, Chicago, USA). The assumption of the normality data was assessed by the Shapiro-Wilk test, the test for the homogeneity of variances using Levene statistics, and the one-way ANOVA test at 0.05 significance to find any difference between the groups.

RESULTS

The result of this study, observing the effect of X-ray radiation towards micronuclei in polychromatic erythrocyte cells of male Wistar rats bone marrow, revealed that the control groups (A1 and B1) had the least number of micronuclei compared to the other groups while the most micronuclei recorded was from A2, followed by B2, A3, and B3 groups (Figure 1).

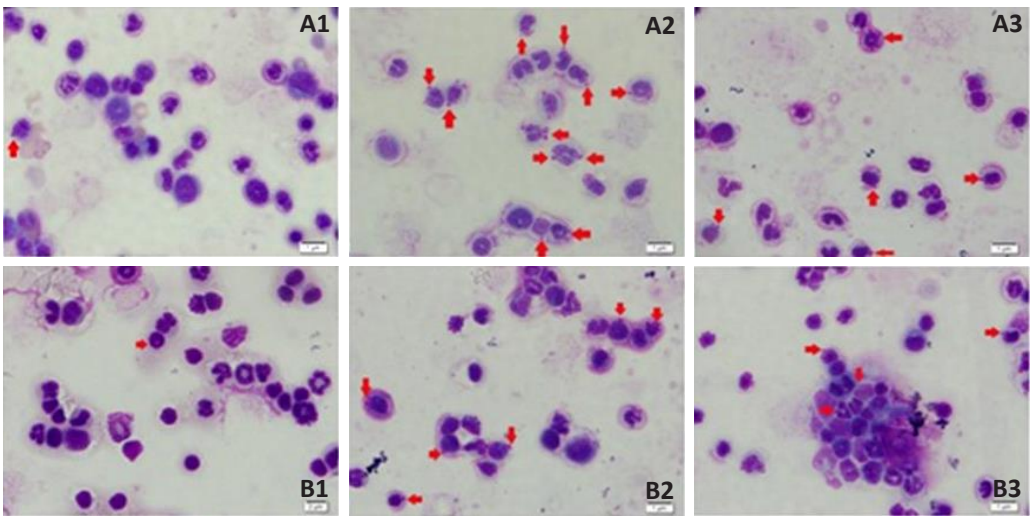

Figure 1. Micronuclei (red arrows) from *polychromatic erythrocyte cells* from each experimental group with 100x magnification.

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Table 1. Mean and standard deviation of micronuclei count on experimental groups 24 hours (A) and 48 hours (B) after radiation exposure

Groups	Micronuclei number Mean ± SD	p-value
A1	3.10 ± 8.75	0.000*
A2	20.30 ± 1.56	
A3	11.30 ± 1.33	
B1	3.40 ± 96	
B2	15.90 ± 87	
B3	6.40 ± 51	

*Significant (<0.05)

Generally, micronuclei count was found less in the groups exposed to both radiation after a 24-hour interlude compared to the groups exposed to both radiations on the same day. The data obtained are presented in mean and standard deviation (Table 1).

Based on those observational results, it can be seen that a time interval between two X-ray exposure results in fewer micronuclei. It is supported by statistical analysis that shows a significant difference between groups ($p<0.05$).

DISCUSSION

Ionizing radiation is known to have an undesirable side effect that results in various changes and damages in the cell.[1] The cell damage, as a consequence of radiation, may be both reversible and irreversible.[9] The damaged cells due to X-ray radiation may recover through a cell repair process, yet it depends on the cell type and radiation dose received.[10] Cells are constantly exposed to internal and external harmful agents such as viruses and chemicals which can lead to changes in cells function and structure. These factors can cause cell necrosis or changes in nucleus genetic information.[11] X-ray is one of the electromagnetic forms that can cause changes in organisms, and it is widely used for diagnosis and treatment in medicine and dentistry.[12] Results of this study show a significant difference in micronuclei cell numbers before and after X-ray exposure. A previous study by Preethi et al.[13] used the cytology method to evaluate the X-ray effects on cells after panoramic and lateral cephalometric radiography. Preethi et al.[13] investigated the genotoxicity effects of panoramic radiography in gingival epithelial cells and showed that X-ray increased genotoxicity in these cells that caused chromosomal damages. Micronucleus index reflects genomic instability, and an increase in the number of micronuclei shows an increased risk for cancer.[7] The damage because of micronuclei formation happens in epithelial basal cells where mitosis happened. Epithelial cell turnover brings them to the surface thus most rates of micronuclei formation happens in mucosal surface one to three weeks after genotoxic factors exposure.[13]

The radiation used in this study was acquired from a single dose panoramic and cephalometric radiograph. A significant increase of micronuclei on polychromatic erythrocyte cells in rats was recorded on groups exposed to both radiations on the same day, observed 24 (A2) and 48 hours (B2) after exposure. The number of micronuclei from both groups was significantly higher compared to both control groups (A1 and B1), which did not receive any radiation exposure.

The micronucleus found in both control groups A1 and B1 denotes micronuclei that are naturally formed without any influence from mutagenic substances exposure.[14] Meanwhile, micronucleus found in treatment groups A2 and B2 are formed due to X-ray exposure from panoramic and cephalometric radiographs. Based on the microscopic examination, it can be seen that in the control group only one to three micronuclei were found in each sample. The result is in accordance with the previous research conducted by Reisz et al.[10] which observed 2000 polychromatic erythrocytes and found about one to two micronuclei in the control group. The formation of natural micronuclei may happen due to several factors such as stress level, which in this study is the experimental animal stress level. Stress may lead to a hypoxic condition that may further affect the production of red blood cells. The red blood cells formation may be impaired or stopped due to cell cycle disruption. Interference in the interphase stage may lead to a decrease in red blood cell production in accordance with the blood cell progenitor sensitivity. Meanwhile, disrupted anaphase or telophase stage may lead to the formation of micronucleus.

Micronuclei formation due to X-ray exposure in panoramic and cephalometric radiography is caused by the ionization process from X-ray which leads to DNA damage and chromosome aberration. The interaction between ionizing radiation and biological substance may give rise to biological side effects immediately after exposure. An X-ray exposure may disrupt the mitotic process by inhibiting the formation of spindle fibres and further causing incomplete chromosome segregation.[7] The micronucleus form in the metaphase-anaphase transition period due to either acentric chromosome fragments or whole chromosome fragments loss during cell division. X-ray exposure may induce the formation of acentric chromosome and chromosome misaggregation. Acentric chromosome fragment and malsegregated whole chromosome fail to interact with spindle fibre, thus, resulting in chromosome instability in the daughter cell. The remaining chromosome fragments will form a micronucleus separated from the daughter cell.[14]

The micronucleus observation in the groups with a 24-hour interlude between panoramic and cephalometric radiograph, both in 24-hours (A3) and 48-hours (B3) observation, are showing significantly fewer micronucleus compared to the groups exposed to both radiations on the same day (A2 and B2). The fewer micronucleus is due to the ionizing radiation from X-ray which may cause a homeostasis process in the cell cycle. The cell cycle may stop, which inhibits cells from entering the G1, S, and G2

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phases, to allow the cell to repair and regenerate, preventing a cell mutation.[15,16] A study by Kalsbeek et al.[17] proved that a delayed cell cycle may result in fewer micronucleus formations.

Based on the result of this study, there is a significant difference in the number of micronucleus in erythrocytes. This result showed that the mature erythrocytes are morphologically more resistant to radiation. The result is supported by previous research, which stated that microscopic damage of erythrocytes will be visible after radiation exposure, yet the mature erythrocytes are more resistant to the exposure.[17]

Another aspect observed in this study is the time interval between the next radiation exposure. A 24-hour interlude can result in fewer micronucleus formations. The micronuclei formed in B3 were significantly lower than A3 groups. This can be caused by the regeneration process of the erythrocyte; an interlude may give chance to the erythrocyte to do a cell repair. This result is in accordance with the previous research which stated that cell damage due to radiation exposure may be reversible through a cell repair process depending on the cell type and the dose of radiation.[10] From the results obtained in this study, it can be concluded that X-ray radiation can affect the formation of micronucleus in polychromatic erythrocyte cells in rats or increase the number of micronucleus in the polychromatic erythrocyte.

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