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The Effect of Periapical Radiography X-Ray Radiation on the Number of Leukocytes in Mice (*Mus musculus*)

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Abstract

BACKGROUND: Periapical radiographic X-ray radiation has ionization energy which can cause cell damage in the body such as damage to the hematopoietic stem cell system in the bone marrow which results in inhibition or cessation of the hematopoiesis process, resulting in a decrease in the number of blood cells, especially leukocytes. A decrease in the number of leukocytes can make the body susceptible to infection with bacteria, viruses, fungi, and other agents that can attack tissues in the oral cavity.

AIM: This study aims to determine the effect of periapical radiographic X-ray radiation on the number of leukocytes in mice (*Mus musculus*).

METHODS: This research is a true experimental study with a posttest-only design with a control group design. The sample in this study was 24 mice, male, bodyweight 25–30 g and age 3–4 months which were divided into four groups, namely, the control group and the treatment group, namely, 1, 7, and 10-times exposure to periapical radiography X-ray radiation.

RESULTS: The results showed that there was a decrease in the leukocyte count of mice at 1, 7, and 10 times of exposure, which was obtained by comparing the leukocyte count of the control group and the treatment group. The number of leukocytes in the control group was 8.16 × 103/μL, the number of leukocytes in the treatment group with 1, 7, and 10 exposures in a row was 7.61 × 10^3/μL, 6.03 × 10^3/μL, and 5.20 × 10^3/μL. The results of statistical tests using One-Way Analysis of variance and *post hoc* Bonferroni showed a significant decrease in the number of leukocytes ($p < 0.05$), namely, in the control group with seven exposures, the control group with ten exposures, and the 1-time exposure group with the 10-time exposure group.

CONCLUSION: There is a decrease in the number of leukocytes in mice due to periapical radiographic X-ray radiation.

Introduction

X-ray radiation in periapical radiographs has ionizing energy that can have effects on the body. Side effects of X-ray radiation can cause inhibition or termination of the process of hematopoiesis, resulting in a decrease in the number of blood cells. X-ray radiation doses below 1 Gy can cause damage to the hematopoietic stem cell system in the bone marrow, but this damage can be repaired by the body within a few days. At higher doses of 2-10 Gy can damage the hematopoietic system severely (bone marrow syndrome) and will be difficult to repair by the body. Damages that occur in the hematopoietic stem cell system can cause a decrease in the number of leukocytes [1], [2], [3].

The normal number of leukocytes in the human body is around 4000–11,000/μL. In this study, using experimental animals in the form of mice (*Mus musculus*) as the object of research where the normal leukocyte count in mice is around 2–10 × 103/μL. An excessive decrease in the number of leukocytes can cause leukopenia. Leukopenia is a condition of decreasing the number of leukocytes in human peripheral blood where the number of leukocytes in the blood is <4000/μL [4], [5], [6]. Leukocytes are white blood cells produced by hematopoietic tissue for the granular type (polymorphonuclear) and lymphatic tissue for the non-granulated (mononuclear) type that function in the body's first defense system against injury or infection. A decrease in the number of leukocytes will make the body more susceptible to infection with bacteria. Viruses, fungi, and other agents can invade tissues. This allows the formation of ulcers in the oral cavity. The bacteria contained in the ulcer will quickly attack the surrounding tissue and if not treated will cause death in less than a week after total acute leukopenia begins [7], [8], [9]. Viral infection begins with local invasion, for example in

the epithelium and mucosa, which can cause herpes. After the virus successfully infects the target cells, immune mechanisms will play a role in controlling the infection. When the immune system decreases due to a decrease in the number of leukocytes, the infection can spread quickly. Fungal infections most often occur in patients who have a weakened immune system, making them susceptible to candidiasis [10].

The peak of the decrease in leukocytes due to X-ray radiation occurred on the first and second days. In the practice of dentistry after surgery, it is highly expected that a good immune system from the patient will help the wound healing process. The decrease in the immune system will be more significant, if the dose of periapical radiography X-ray radiation is absorbed by the patient more. In taking pictures of teeth with periapical radiographs, errors can occur both due to technical and processing so that it is necessary to do repetition. This repetition will subject the patient to re-exposure thereby increasing the radiation dose absorbed by the patient.

Based on the description of the background above, the researchers wanted to examine the effect of X-ray radiation on periapical radiographs on the decrease in the number of leukocytes in mice. In one periapical radiograph, the resulting dose is 0.95 mGy, for 7 repetitions the dose will be 6.64 mGy, and for 10 repetitions the resulting dose is 9.54 mGy. In this study, periapical radiographic exposure (irradiation) was performed 1, 7, and 10 repetitions. This is because in this study the researchers wanted to determine the effect of periapical radiography X-ray radiation on the number of leukocytes in mice (*M. musculus*) given a dose below 7 mGy, namely, with one irradiation, with doses approaching and above 7 mGy, respectively, 7 and 10 irradiation times.

Research Materials and Methods

This research conducted at Gusti Hasan Aman Dental and Oral Hospital Banjarmasin, Banjarmasin Kalimantan Superior Polytechnic Laboratory and Veterinary Center (BVET) Regional V Banjarbaru in January-March 2019. This study uses a true experimental design which is an experimental laboratory and was carried out *in vivo* with experimental animals, namely, male mice (*M. musculus*). This research design uses post-test only with control group design with the consideration that each sample has the same characteristics before being given treatment. In addition, technically the initial measurement is not possible because it will damage the sample. The population of this study was male (*M. musculus*) mice with the following sample criteria:

Inclusion criteria

The following criteria were included in the study:

1. Male mice (*M. musculus*)
2. 3–4 months old
3. Body weight 25–30 g
4. In good health (active and have a good appetite).

Exclusion criteria

The following criteria were excluded from the study:

1. Mice die
2. Mice are not normal (there are wounds or defects)
3. Mice are in an unhealthy state such as weak, inactive and have no appetite
4. There is a weight loss of more than 10% after the adaptation period in the laboratory.

Research procedure

1. Test Animal Preparation
   Experimental animals were adapted for 7 days by being given food to obtain sample uniformity before conducting research to control experimental animals
2. Experimental Animal Grouping
   The 24 experimental animals were grouped randomly into four groups, namely:
   a. Group I: no treatment (control group)
   b. Group II: X-ray radiation exposure of periapical radiographs 1 time
   c. Group III: X-ray radiation exposure of periapical radiographs 7 times with a repetition time difference of 1 min
   d. Group IV: X-ray radiation exposure of periapical radiographs 10 times with a repetition time difference of 1 min
3. Placement of Experimental Animals When Radiated
   Under the cage that has been put in the mice, a dosimeter is placed and the radiation irradiation focuses on the direction of the head of the mice
4. Radiation Exposure
   Radiation exposure was given to the head area of the experimental animal at a distance of 30 cm from the ASAHI brand dental periapical radiography unit which has an electric voltage of 60 kV and an electric current of 10 mA in 0.63 s. The resulting dose for 1 time exposure to periapical radiographic X-ray radiation is 0.95 mGy. The exposure was carried out 1, 7, and 10 times with a repetition time interval of 1 min

5. Experimental Animal Transport Techniques
   The technique of transporting experimental animals from the Gusti Hasan Aman Hospital in Banjarmasin to the Banjarbaru Veterinary Center by car
6. Blood Sampling
   Blood sampling in mice was carried out after 24 h of radiation. Mice to be sacrificed for blood collection were given inhalation anesthesia using 5 ml of diethyl ether. The blood that has been taken is placed in a microtube which already contains 0.01 ml of 10% EDTA
7. Leukocyte Count Procedure
   The leukocyte count was calculated using a hematology analyzer
8. Handling of experimental animals after sampling is done
   The burial was carried out in the backyard of the Regional V Regional Veterinary Laboratory (BVET) Banjarbaru which is a special place for the burial of experimental animals
9. Data collection and collection procedures
   The data obtained in this study are primary data. The data collected were then recorded
10. Method of data processing and analysis
    The data were first tested for normality using the Shapiro-Wilk and homogeneity test with the Levene test. The results of data analysis showed that the data were normally distributed and the variance was the same, so parametric statistical tests were carried out using one-way analysis of variance with a 95% confidence level (α = 0.05%) and to determine the significance value followed by the *post-hoc* Bonferroni test. Data processing in this study was processed using the SPSS type 22 computer program.

Results

In this study, radiation exposure was given to the head area of experimental animals at a distance of 30 cm from the ASAHI brand dental periapical radiography unit which had an electric voltage of 60 kV and an electric current of 10 mA in 0.63 s. Periapical radiographic X-ray radiation dose (mGy) was measured using a dosimeter. The resulting dose for 1-time exposure to periapical radiographic X-ray radiation is 0.95 mGy. The exposures were performed 1, 7, and 10 times with a repetition time of 1 min. The calculation of the leukocyte count of mice (μL) was obtained using a hematology analyzer after 24 h of radiation exposure.

Table 1 shows the average dose of periapical radiographic X-ray radiation absorbed by mice (M. musculus) after administration of 1, 7, and 10 times exposure to periapical radiographic X-ray radiation, respectively, namely, 0.95 mGy, 6.64 mGy, and 9.54 mGy.

Table 1: Average absorbed periapical radiography dose in mice

Total radiation exposure X-ray radiography periapical	Mean exposure (mGy)
B	0.95
C	6.64
D	9.54

A: 1 time exposure, B: 7 repetitions of exposure, C: 0 repetitions of exposure.

Table 2 shows the average decrease in the number of leukocytes in mice (*M. musculus*) in the untreated control group and the treatment group, namely, 1, 7, and 10 repetitions of exposure after 24 h of exposure to periapical radiographic X-ray radiation.

Table 2: Average (Mean and Standard Deviation) leukocyte count due to X-ray periapical radiographs with an electric voltage of 60 kV and an electric current of 10 mA within 0.63 s

Group	n	Mean ± Standard deviation scoring ($\times 10^3$/L)
A	6	8.167 ± 1.1994
B	6	7.617 ± 0.9745
C	6	6.033 ± 1.1325
D	6	5.200 ± 0.8532

A: Control, B: 1 time exposure, C: 7 repetitions of exposure, D: 10 repetitions of exposure.

Figure 1 diagram of the average leukocyte count of mice showing a decrease in the number of leukocytes in mice after exposure to X-ray radiation on periapical radiographs. This shows that the more exposure to periapical radiography X-ray radiation is given, the more the decrease in the number of leukocytes will occur.

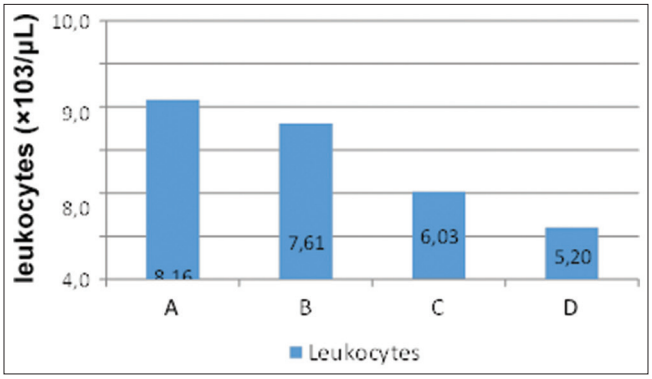

Figure 1: Diagram of the Average Leukocyte Count of Mice
Description: A: Control, B: 1 time exposure, C: 7 repetitions of exposure, D: 10 repetitions of exposure

Table 3 shows that there is no significant difference (p > 0.05) between Group A and Group B, Group B and Group C, and Group C and Group D. The group which shows that there is a significant difference (p < 0.05), namely, Group A with Group C, Group A with Group D, and Group B with group D.

Table 3: Bonferroni statistical test results for the number of leukocytes in mice

Group	A	B	C	D
A		1,000	*0.013	*0.001
B	1.000		0.099	*0.004
C	*0.013	0.099		1.000
D	*0.001	*0.004	1.000	

*There is a significant difference (p < 0.05), A: Control, B: 1 time exposure, C: 7 repetitions of exposure, D: 10 repetitions of exposure.

Discussion

This study was conducted with the aim of proving a decrease in the number of leukocytes in mice (*M. musculus*) due to periapical radiographic X-ray radiation. The results showed that there was a decrease in the number of leukocytes in mice after being given treatment in the form of 1, 7, and 10 repetitions of periapical radiography X-ray radiation exposure in 0.63 s with a repetition time interval of 1 min. The decrease in the number of leukocytes that occurred can be seen by comparing the number of leukocytes of mice in the control group with the treatment group. The number of leukocytes in the control group was 8.16×10^3/μL which was compared to the number of leukocytes in the 1-time exposure group which was 7.61×10^3/μL, the number of leukocytes in the group given 7 exposures was 6.03×10^3/μL, and the number of leukocytes in the group that was exposed to 10 times was 5.20×10^3/μL.

The decrease in the number of leukocytes that occurs determines the level of the effect of X-ray radiation which depends on the dose absorbed by the body. The more doses the patient absorbs, the more the decrease in the number of leukocytes occurs, therefore BAPETEN has set a limit on the dose that a patient may absorb for intraoral dental examinations at 7 mGy. In this study, there was a non-significant decrease in the number of leukocytes between the control group and the 1-time exposure group, this is because the dose produced by the periapical radiograph is only 0.95 mGy and is still far from the threshold that has been set by BAPETEN, which is 7 mGy [4], [11].

The body that received a higher dose of radiation and the number of re-exposure to X-ray radiation will cause a decrease in the number of leukocytes more than the lower dose. This is also evidenced by the research of Shanshoury *et al.* (2016) which showed there was a significant decrease in the number of leukocytes in mice that received a larger radiation dose, namely the number of leukocytes in the control group of 9.99×10^3/μL, it was found that there was a decrease in the number of leukocytes in the control group. With a dose of 0.1 Gy of 9.41×10^3/μL and there was a significant decrease in the number of leukocytes in the group with a dose of 0.5 Gy of 6.47×10^3/μL [12]. The decrease in the number of leukocytes that occurs is caused by the radiosensitivity of leukocytes which is higher than other blood cells due to their function as the body/s defense system. The high level of cell radiosensitivity is determined by the level of proliferation, the higher the proliferation or cell division that occurs, the more sensitive the cell will be. Leukocyte cells when exposed to radiation can die because X-ray radiation has ionizing energy which if given in excess can cause damage to the tissues it passes through [4], [13], [14], [15]. The higher the proliferation or cell division that occurs, the more sensitive the cell will be. Leukocyte cells when exposed to radiation can die because X-ray radiation has ionizing energy which if given in excess can cause damage to the tissues it passes through [4], [13], [14], [15].

The decrease in the number of leukocytes in this study was caused by cell damage due to the ionization energy of X-ray radiation. It is also mentioned in previous studies that the cell damage that occurs is the result of biological effects indirectly or directly from the X-ray radiation received by the patient. Both of these effects can affect the DNA of a cell which can cause damage by breaking one DNA strand (SSB) or breaking two DNA strands (DSB). Damages to cells that occur can be repaired physiologically without errors so that the DNA structure can return to its original state, which is where the starting point of repair begins on the 3rd day after radiation [16], [17]. This repair process goes through several stages, namely, detection of damaged DNA strands, cutting of damaged DNA strands, synthesis of repair of damaged DNA strands, and the process of connecting new DNA strands by ligase. In the process of DNA repair using SSB, UvrABC protein is needed, which is an enzyme complex consisting of uvrA, uvrB, and uvrC subunits.

In the early stages of repair, uvrA and uvrB will bind to detect damage to the DNA strand. UvrAB which has detected DNA damage will result in the release of uvrA in the uvrAB bond and urvB remains in the area of DNA damage. After that, uvrB will bind to uvrC to become uvrBC to cut DNA nucleotides that have been detected as damaged. The nucleotide cleavage of the damaged DNA causes the release of urvC and the loss of the damaged part with uvrB. The next process is continued by the polymerase II enzyme to re-synthesize the part of the nucleotide that was damaged. In the final stage of repair, the synthesized nucleotides are incorporated into the DNA strand by the ligase enzyme [18], [19].

Under certain conditions, such as the breaking of two DNA strands (DSB) due to the influence of X-ray radiation, it will be difficult to repair because in the repair process an error occurs, causing cell death that triggers apoptosis in the HSC system [1], [16]. X-ray radiation that causes DSB DNA damage in HSC will be detected by the RAD50 protein located on the ribosome. After detecting the damage, the RAD50 protein will activate Ataxia Telangiectasia Mutated (ATM). ATM functions to activate Checkpoint kinase 2 (CHK2), in which CHK2 will work by stopping the process of cell mitosis and examining damaged cells. ATM will also activate P53 to activate the p53 upregulated modulator of apoptosis (PUMA). PUMA works in two ways, namely,

by activating proapoptotic proteins such as Bax and Bak, then blocking the action of antiapoptotic proteins such as BCL-2 and BCL-x. Proapoptotic proteins have been active cause disruption of the integrity of the cell's mitochondria so that initially cytochrome-c binds to the mitochondria and releases and binds to Apaf-1. The bond between cytochrome-c and Apaf-1 will activate caspase 9 and end in caspase 3 activation. Activation of caspase 3 will cause endonuclease proteins to fragment DNA and cause proteases to degrade the cell nucleus and cell cytoskeleton. The degradation of the cell nucleus will cause fragmentation of the cell nucleus and the degradation of the cytoskeleton causes the cell to lose its cell integrity. Resulting in apoptosis which is characterized by the formation of apoptotic bodies. Apoptosis in the HSC system causes disruption of the leukocyte formation process which results in a greater decrease in the number of leukocytes in the body [17], [20].

In this study, the number of leukocytes in mice with 1 time exposure to periapical radiography X-ray radiation was 7.61 × 10^3/μL, the number of leukocytes in mice with seven exposures to periapical radiography X-ray radiation was 6.03 × 10^3/μL, and the number of leukocytes in mice with 10 times exposure to periapical radiography X-ray radiation was 5.20 × 10^3/μL. The leukocyte count of mice with 1, 7, and 10 repetitions of exposure still showed the number of leukocytes within normal limits because mice had a normal leukocyte count of 2–10 × 10^3/μL. Statistically the administration of 7 and 10 repetitions of exposure where the dose was close to and above 7 mGy showed a significant decrease in the number of leukocytes. This can be a consideration for dentists before performing surgery due to a decrease in the number of leukocytes where the immune system will decrease which can cause infections to spread quickly. To avoid this, it is necessary to set limits on the frequency of repetition of periapical radiography in patients. Based on this study, the maximum limit for taking periapical radiographs was 7 times. It can also provide an overview, information, and knowledge to the public that performing a periapical radiograph is not as dangerous as radiotherapy and the effects after taking periapical radiographs do not result in manifestations of leukopenia such as bacterial, viral, or viral infections.

The obstacle faced in this study was the behavior of mice (*M. musculus*) which were sometimes active during preparation before giving periapical X-ray radiation exposure. In this study, the use of mice was only used to describe how the effect of periapical radiographic X-ray radiation on the number of leukocytes cannot be equated with its effect on humans; mice were used in this study because mice have a physiological system similar to humans.

Conclusion

Based on the research that has been done, it can be concluded that there is a decrease in the number of leukocytes in mice (*M. musculus*) due to X-ray radiation of periapical radiographs.

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