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The Effect of Periapical Radiography X-Ray Radiation on Platelets, Leukocyte, Hemoglobin and Mean Corpuscular Volume (MCV) in Mice (*Mus Musculus*)

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Abstract

Periapical radiographs are intraoral radiographs that can provide clearer and more detailed information about the teeth and surrounding tissue. Based on the recommendations of the *International Actomic Energy Agency* (IEIA) and the *Nuclear Energy Supervisory Agency* (BAPATEN), the dose level for intraoral dental examination is 7 mGy. X-ray radiation produced by periapical radiographs can cause cell damage in the body, which one of the cells that is damaged is hematopoietic cells in the bone marrow.

Purpose: The purpose of this study was to prove a decrease in thrombocytes (platelets), leukocytes, hemoglobin count and the value of *Mean Corpuscular Volume* (MCV) in mice (*Mus musculus*) due to X-ray radiation periapical radiographs.

Method: This study was a true experimental design with a post test only with control group design that was given 1, 7, and 10 times the exposure of periapical radiographs of X-ray radiation in the treatment group then compared to the untreated control group. The measurement of the dose absorbed by the mouse in this study was carried out using dosimeters, and after 24 hours the treatment was given, it is then calculated the number of mouse leukocytes by using a hematology analyzer.

Results: The results showed a decrease in blood in the first exposure group, which are platelets 713 x103sell / µL, leukocytes 7.61 × 103 / µL, hemoglobin 13.71 g / dL, and MCV 52.967 fl. The decrease in the 7-exposure group was platelets 699.5x103sell / µL, leukocytes 6.03 × 103 / µL, hemoglobin 12.81 g / dL, and MCV 52.167 fl. The decrease in the 10-exposure group was platelets 688,833 x103sell / µL, leukocytes 5,20 × 103 / µL, hemoglobin 12,06 g / dL, and MCV 51,483 fl. Thus, it can be concluded that the decrease in number that occurs in mouse is still within the normal limits.

Keywords: *Periapical radiographs, platelets, leukocytes, hemoglobin, MCV.*

Introduction

Periapical radiographs produce X-ray radiation which can cause ionization in body cells. Ionization can cause cell damage in the body, one of the cells that is damaged is hematopoietic cells in the bone marrow.[1,2] Radiation doses of 1-10 Gy result in severe hematopoietic cell damage and hematopoietic cells that have a difficult damage to repair. X-ray radiation less than 1 Gy can also result in hematopoietic cell damage, but hematopoietic cells that experience the damage can be repaired.[3,4,5,6]

Decreased platelets and leukocytes due to X-ray radiation occur on the first and second day after radiation.[3,5,11] Dentists who carry out invasive actions in thrombocytopenia patients can increase the risk of prolonged bleeding. As well as post surgery, a good immune system is expected from the patient to help the wound healing process. The results of this study are aimed at making dentists more careful in carrying out invasive actions to patients after one and two days of periapical radiograph radiographs, and reducing the risk of developing oral disorders such as SAR.[9,12,13]

Patients who receive a larger dose of X-ray radiation will experience a greater effect on the body, therefore the International Atomic Energy Agency (IAEA) and the Nuclear Energy Supervisory Agency (BAPETEN) make limits on doses that can be absorbed by patients for intraoral radiographic examination is 7 mGy. In fact, many patients repeat repetition of periapical radiographs. Repeated shooting can result in larger patients receiving radiation doses, even patients can receive doses above 7 mGy.[4,14,15,16]

Based on the description of the background above, the researcher wanted to examine the effect of periapical radiographs of X-ray radiation on the decrease in platelet counts, leukocytes, hemoglobin, and MCV in mice.

Materials and Method

This study uses a true experimental research design by using post-test only with control group design. The samples in this study were 24 male mice with the age of 3-4 months and 25-30 grams of body weight. The material used in this study is a 5 ml Diethyl ether and anti-coagulant in the form of 10% EDTA. The tool used during this study was the dental radiographic unit brand ASAHI with a power supply of 60 kv electric current of 10 mA, dosimeter, wire cage to put mice when radiographed, 1 ml syringe, micro tube which already contained 10% EDTA as much as 0.01 ml, mice maintenance cages, eating places and mice drink bottles, hematology analyzers and stopwatch.

In the first stage of the study, mice were adapted for 7 days by being fed BR2 *Comfeed* and standard distilled water. The adaptation that was carried out, then followed by grouping mice randomly into 4 groups, namely the control group, group 1 X-ray radiation exposure, group 7 exposure to X-ray radiation, and group 10 times the exposure to X-ray radiation.

The dose produced at one time X-ray radiation is 0.95 mGy. The exposures were carried out 1.7 and 10 repetitions with a distance of 1 minute interval in each repetition. Mice that have been carried out X-ray radiation at the Gusti Hasan Aman Hospital RSGM Banjarmasin will be taken to the Banjarbaru Veterinary Center for blood sampling.

24 hours after X-ray radiation, it is anesthetized by inhalation in mice using 5 ml diethyl ether. Mice that have been anesthetized then, taken blood in the heart of mice using 1 ml syringe. The blood sample that has been obtained will be placed in the microtube in which there is 10% EDTA as much as 0.01 ml. The blood samples that have been obtained will be carried out a complete blood test using the hematology analyzer, and the results of the data obtained will get parametric tests using One Way Anova.

Results

When doing X-ray radiation in mice, the researcher also calculated the doses amount of X-ray radiation absorbed by mice using dosimeters. The number of radiation doses received by mice at 1 time X-ray radiation exposure is 0.95 mGy, the dose received by mice at 7 times exposure to X-ray radiation is 6.64 mGy, and the dose received by mice at 10 times exposure to X-ray radiation is 9.54 mGy. Twenty-four hours after X-ray radiation periapical radiographs in mice, blood was taken to measure platelet counts, leukocytes, hemoglobin and MCV of mice.

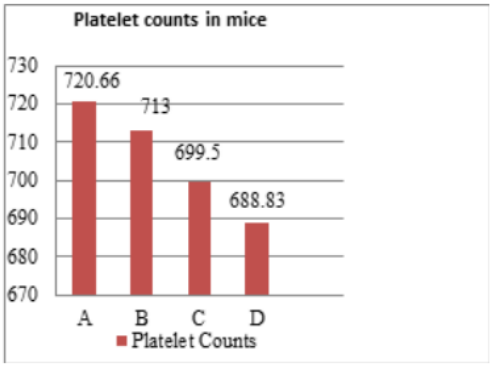

Fig 1. Platelet counts in mice in each treatment

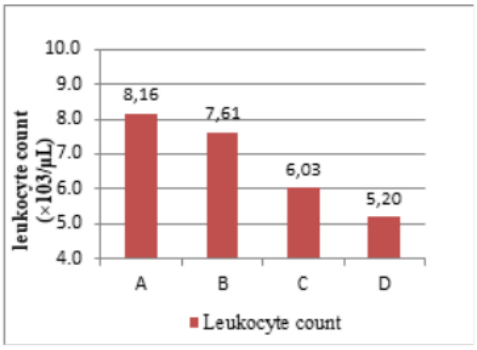

Fig 2. Leukocyte counts in mice in each treatment

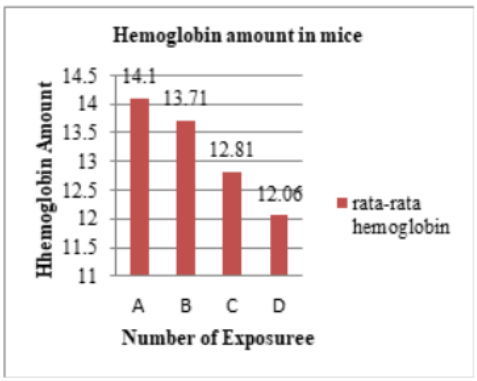

Fig 3. Hemoglobin amount in mice in each treatment

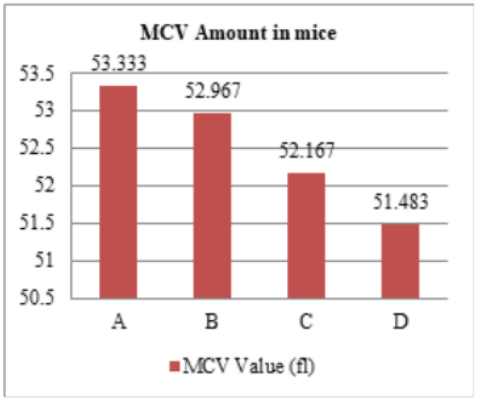

Fig 4. Hemoglobin amount in mice in each treatment

Information:

A : Control

B : 1 time exposure

C : 7 repetitions of exposure

D : 10 repetitions of exposure

In Figures show that there is a decrease in platelet count, hemoglobin and MCV leukocytes after mice receive X-ray radiation. The greater the frequency of exposure to periapical radiographs of X-ray radiation, the greater the decrease in platelets, leukocytes, hemoglobin and MCV in mice. This shows that the more the frequency of repetition of periapical radiographs, the greater the effect caused to the body.

The results of the data on the average platelet, then a normality test was performed using Saphiro-Wilk. The results of the normality test show that the data is normally distributed. This is because in each treatment $p > 0.05$ with a significance value in the control group is 0.365, in the group 1 time exposure is 0.571, in the group 7 times exposure is 0.361 and in the group 10 times exposure is 0.51. The results of the study were continued with homogeneity tests with Leven's test. In the homogeneity test shows that the data is distributed homogeneously with a significance value of $p = 0.124$.

The results of the data on the average number of leukocytes, then a normality test was performed using Saphiro-Wilk. The results of the normality test show that the data is normally distributed. This is because in each treatment $p > 0.05$ with a significance value in the control group is 0.456, in the group 1 time exposure is 0.632, in the group 7 times exposure is 0.415 and in the group 10 times exposure is 0.349. The results of the study with a significance value of $p = 0.909$

The results of the data on the average amount of hemoglobin then a normality test was performed using Saphiro-Wilk. The results of the normality test show that the data is normally distributed. This is because in each treatment $p > 0.05$ with a significance value in the control group is 0.866, in the group 1 time exposure is 0.326, in the group 7 times exposure is 0.848 and in the group 10 times exposure is 0.955. The results of the study were continued with homogeneity tests with Leven's test. The homogeneity test shows with a significance value of $p = 0.265$

The results of the data on the average number of MCVs, then normality tests were performed using Saphiro-Wilk. The results of the normality test show that the data is normally distributed. This is because in each treatment $p > 0.05$ with a significance value in the control group is 0.588, in the group 1 time exposure is 0.799, in the group 7 times exposure is 0.752 and in the group 10 times exposure is 0.664. The results of the study were continued with homogeneity tests with Leven's test. The homogeneity test shows that the data is distributed homogeneously with a significance value of $p = 0.866$

Data that has been normally distributed and homogeneous will be continued with the One Way ANOVA parametric test with a confidence level of 95% ($\alpha = 0.05$) to determine whether there is a difference in the spread of data. The One Way ANOVA test results obtained $p = 0{,}000$, which means that there are significant differences between the treatment groups. Furthermore, the data analysis was continued by a follow-up test, the Bonferroni Post Hoc test.

Providing the results of *Post Hoc Bonferroni, it* can be seen that in the control group there were significant differences with the group 7 times exposure to X-ray radiation, and the group 10 times exposure to X-ray radiation. One group of X-ray radiation exposures showed significant differences with the group 10 times exposure to X-ray radiation.

Discussion

The decrease in platelet counts, leukocytes, hemoglobin and MCV in this study occurred because of the damage to *Hematopoietic stem cells* (HSC) in the bone marrow. Periapical radiographs of X-ray radiation produce ionization energy.[5,17,18]

X-ray radiation that causes HSC DNA damage in SSbs is easy to repair, so the HSC will return to normal. SSCs undergoing HSB damage will go through several stages of the repair process including detection of damaged DNA strands, cutting of damaged DNA strands, damaged DNA strand synthesis, and the process of connecting new DNA strands. The SSbs protein repair process requires UvrABC protein.[19]

X-ray radiation that damages HSC DNA by DSBs will be detected by the RAD50 protein. The RAD 50 protein that has detected damage will activate the Ataxia Telangiectasia Mutated (ATM). The ATM serves to activate Checkpoint kinase 2 (CHK2), CHK2 will work by stopping cell mitosis and carrying out checks on damaged cells. ATM also activates P53, then the activated P53 will activate the upregulated modulator of apoptosis (PUMA) p53 to begin the apoptosis process. PUMA works in two ways, namely by activating proapotosis proteins such as Bax and Bak, and blocking the work of antiapoptotic proteins such as BCL-2 and BCL-x. Proapoptotic proteins that have been active will cause disruption of the integrity of mitochondrial cells, so that cytochrome-c which initially binds to mitochondria will be released and bind to Apaf-1. The bond between cytochrome-c and Apaf-1 will cause caspase 9 activation and end in Activation of caspase 3 will cause endonuclease proteins to fragment DNA, besides that caspase 3 also causes proteases to degrade cell nuclei and cell cytoskeleton. The nucleus and cytoskeleton of cells that have been damaged will cause cells to experience apoptosis which will eventually form apoptotic bodies.[14.17,21] Apoptosis that occurs in HSC results in disruption of blood cell production in the body which results in decreased platelet counts, leukocytes, hemoglobin and MCV in the body's circulation.[22,23,24,25]

The results of this study indicate that there is a decrease in platelet count, leukocytes, hemoglobin and MCV values that are not significant in the group 1 time radiation exposure with the dose produced at 1 time exposur is 0.95 mGy, the dose of 0.95 mGy is still far below the dose limit set by BAPETEN. There was a decrease in platelet count, leukocytes, hemoglobin and a significant MCV value in the group given 7 times exposure to X-ray radiation with the dose produced at 7 times exposure was 6.64 mGy. The decreased platelet count, leukocytes, hemoglobin and significant MCV values were also seen in the group 10 times exposure to X-ray radiation with the dose produced at 10 exposures was 9.54 mGy, the dose had exceeded the radiation dose limit set by BAPETEN. The decrease in platelet value, leukocytes, hemoglobin and MCV values in this study were compared with the control group.[4,14,15]

That increasing the dose of X-ray radiation periapical radiography can cause a decrease in the number of leukocytes that are increasingly significant. Showed a significant reduction in leukocyte counts in rats receiving larger radiation doses, namely the number of rat leukocytes in the control group at 9.99×103/µL, the number of leukocytes in the group with the dose of 0.1 Gy was 9.41×103/µL and the number of leukocytes in the group with a dose of 0.5 Gy was 6.47 × 103 / µL. This is because mice have a normal leukocyte count of 2-10×103/µL. Statistically the administration of 7 and 10 repetitions of exposure with doses absorbed close to and above 7 mGy showed a significant reduction in leukocyte count. This study remains to be minimized because the decrease in hemoglobin and MCV is the trigger factor for recurrent aphthouse stomatitis (RAS) which is one of the ulcers in the oral cavity that causes pain.[24]

In this study it can provide information to the public that the effects after x-ray periapical radiographs are not as dangerous as the effects caused by radiotherapy. Based on this study stated that 7 times the frequency of x-ray repetition periapical radiographs is the maximum frequency limit that can be done to patients, it would be better if the frequency of radiograph periapical radiograph repetition was given to patients less than 7 repetitions.[15,16]

Source of Funding: Domestic government

Conflict of Interest: There is no conflict of interest in this study.

Ethical Clearance: This study obtained a label of ethics escaped by the number:136/KEPKG-FKGULMM/EC/I/2019 on Januari 2019.

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