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**THE EFFECT OF PANORAMIC RADIOGRAPHY ON THE NUMBER OF  
MICRONUCLUES IN PERIODONTITIS**

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**ABSTRACT**

**Background:** Periodontitis is a periodontal disease that can cause an increase in Reactive Oxygen Species (ROS). The increase in ROS concentration will cause oxidative stress which has a role in DNA damage. In the case of periodontitis, panoramic radiography plays an important role in showing generalized horizontal bone loss. Radiation on panoramic radiography may cause DNA damage. DNA damage that occurs due to periodontitis or panoramic radiographic radiation exposure is characterized by the formation of micronuclei in gingival epithelial cells. **Objective:** To determine the effect of panoramic radiography on the number of micronuclei in Wistar rats with periodontitis. **Method:** This study was true experimental with post-test only and control group design. This study used 15 male Wistar rats which divided into 5 groups. **Result:** The average number of micronuclei in the normal group and 1 time exposures was 4 compared to the periodontitis group without exposure was 1.67, periodontitis and 1 time exposures was 8, periodontitis and 2 times the exposure was 15.67, and in the periodontitis and 3 times the exposure was 42.67. Result of One-Way Annova test and Post Hoc Bonferroni test indicated that significant changes in the number of micronuclei was seen between the normal group with 1 time exposure to the periodontitis group with 2 times and 3 times exposure and in the periodontitis group without exposure to the periodontitis group with 2 times and 3 times exposure. **Conclusion:** Panoramic radiograph X ray radiation and periodontitis can cause changes in the number of micronuclei in wistar rats.

**Keywords:** , Micronucleus, Panoramic Radiography, Periodontitis.

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**INTRODUCTION**

Periodontitis is a periodontal disease that can cause the formation of Reactive Oxygen Species (ROS).<sup>1,2</sup> Types of ROS such as superoxide anion, hydroxyl radical, nitrous oxide and hydrogen peroxides are produced through the bacteria host interaction mediated pathway, stimulating PMN leukocytes to produce superoxide radicals through respiratory burst.<sup>1</sup> This action results in an increase of ROS concentration, leading to oxidative damage to the periodontal tissues. This increase in oxidative stress will play a role in the destruction of periodontal tissue.<sup>1</sup>

Beside having a role in the destruction of periodontal tissue, oxidative stress also plays a role in causing DNA damage.<sup>1</sup> One method to determine DNA damage is the micronucleus assay.<sup>1</sup> Tadin *et al* (2019) mentioned that DNA damage is more common in individuals with

periodontitis than individuals without periodontitis.<sup>3</sup>

In periodontitis, panoramic radiography plays an important role in helping to determine the prognosis and treatment plan to assess the severity of alveolar bone damage and can show generalized horizontal bone loss from mild to moderate.<sup>4,5</sup> Panoramic radiography in periodontitis patients is usually used twice, before and after treatment to evaluate treatment results.<sup>5</sup>

Panoramic radiography can cause formation of micronucleus due to radiation exposure which has a biological effect in the form of cell damage through oxidation reactions that produce free radicals.<sup>6</sup> Radiation exposure will be absorbed inside the body so it can cause biological effects on body cells.<sup>7</sup> The size of side effects or complications obtained during patients using panoramic radiography is influenced by several

factors, one of the factors is the radiation dose.<sup>8</sup> The radiation dose used in panoramic radiography can cause DNA damage.<sup>9</sup> DNA damage is characterized by formation of the micronucleus.<sup>10</sup> Sheikh *et al* (2012) and Saputra *et al* (2019) mentioned that micronucleus formed on gingival epithelial cells after panoramic radiography and the largest number of micronucleus occurred on the 7<sup>th</sup> day after exposure to panoramic radiography.<sup>6,11</sup>

Micronucleus comes from chromosome fragments or all chromosomes left during the division of the nucleus (anaphase) and found in cells with DNA damage.<sup>1,12</sup> Micronucleus functions as a biomarker that provides information about cytogenetic damage in human tissue that is potentially affected by carcinogens.<sup>13</sup> Shatingsih *et al* (2013) mentioned that the number of micronucleus can increase in epithelial cells in the gingival mucosa.<sup>10</sup> Increased micronucleus occurs due to migration of cells containing micronucleus in the basal stratum, then differentiate and keratinize towards a more superficial layer, so the micronucleus can be found in the mucosal epithelium.<sup>14</sup>

Research about the number of micronucleus on the effects of X-ray on panoramic radiography and in periodontitis have done a lot. However, no one has combined between the periodontitis and X-ray panoramic radiography observed through the number of micronucleus. In periodontitis, especially with generalized bone loss, it is a must to do a panoramic radiography examination, therefore research is needed to find out whether in a periodontitis that uses panoramic radiography can increase the number of micronucleus or not. In addition, BAPETEN (Badan Pengawas Tenaga Nuklir) 2019 mentioned that patients do not need dose restrictions as in NBD (Dose Limit Value). However, the dose received by the patient must be justified and optimized to prevent the reception of unnecessary radiation exposure.<sup>15</sup> Therefore, the researchers wanted to examine the effect of panoramic radiography on the number of micronucleus in wistar rats with periodontitis that were given 1 time, 2 times, and 3 times the repeated panoramic exposure.

## MATERIAL AND METHODS

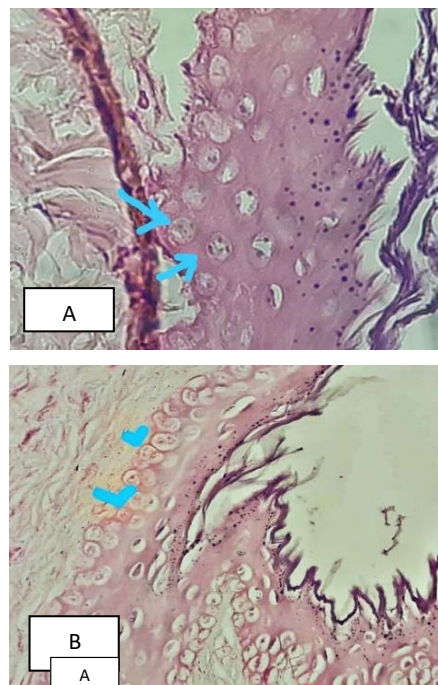
This research has passed the ethical clearance issued by Ethics Committees Faculty of Dentistry, Lambung Mangkurat University Banjarmasin No.076/KEPKG-FKGULM/EC/I/2020. This research was done in Laboratory of Balai Veteriner Banjarbaru, Departement of Radiology, Idaman Hospital, Banjarbaru and Laboratory of Anatomic Pathology, Ulin Hospital, Banjarmasin.

This study used a true experimental method with control group design. The sample of this study were 15 male wistar rats aged 1,5-2 months and weight 150-200 gram divided into 5 groups, namely normal and 1 time of X ray radiation exposure, periodontitis without X ray radiation exposure and periodontitis with 1, 2, and 3 times X ray radiation exposure.

The first step was adaptation the wistar rats for 7 days to obtain uniformity of the sample before treatment. Wistar rats that have been adapted were carried out by the induction of *Porphyromonas gingivalis* for 2 weeks until they showed clinical signs of periodontitis. After wistar rats periodontitis, X-ray radiation was carried out using a panoramic radiograph of the Villa Rotograph EVO brand with an electric voltage of 70 kV, an electric current of 60 mA and within 14 seconds. After a panoramic radiograph X-ray exposure, wistar rats were re-adapted for 7 days and then did a removal of the gingival tissue of the wistar rat to see the micronucleus. Interpretation and calculation of micronucleus were using a light microscope with a magnification of 400x at 5 visual fields.

## RESULTS

The histopathological results of a micronucleus examination in gingival wistar rats can be seen in these pictures.



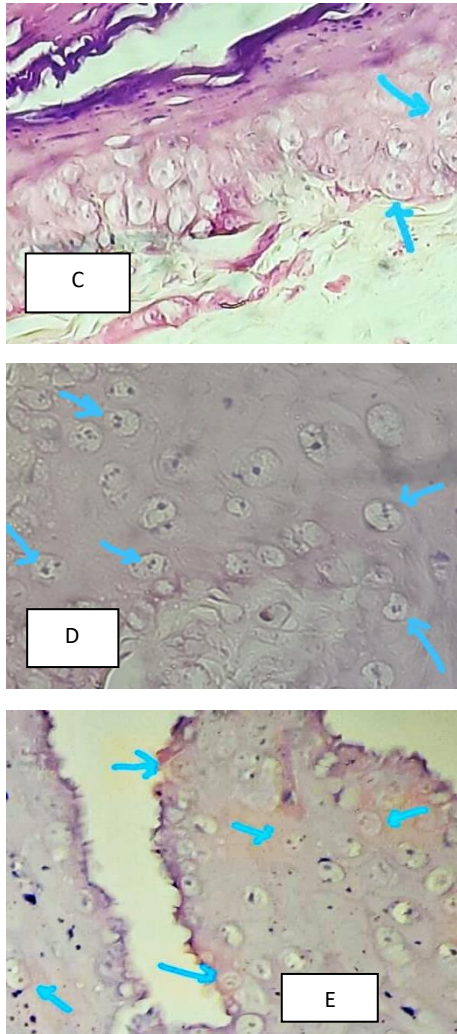


Figure 1. Histopathology of micronucleus on gingival epithelial cell of wistar rats. Figure A is the normal group with 1 time exposure, figure B is periodontitis without exposure, figure C, D, E are periodontitis group with 1, 2, and 3 times exposure. In each group a micronucleus is seen with the character of an additional smaller nucleus, located around the nucleus in gingival epithelial cells of male wistar rats (*Rattus novergicus*) which was observed using a 400x magnification light microscope with 5 visual fields.

Seven days after panoramic radiographic radiation exposure, the gingival tissue of the wistar rats was removed. The mean value of micronucleus in all groups can be seen in table 1.

Table 1. The Average Number of Micronucleus In Wistar Rats.

Group	N	Mean ± SD Number of Cell
Normal and 1 Time Exposure	3	4,00 ± 1,000
Periodontitis Without Exposure	3	1,67 ± 1,528
Periodontitis and 1 Time Exposure	3	8,00 ± 1,000
Periodontitis and 2 Times Exposure	3	15,67 ± 1,528
Periodontitis and 3 Times Exposure	3	42,67 ± 7,024

Figure 2. The average number of micronucleus in wistar rats.

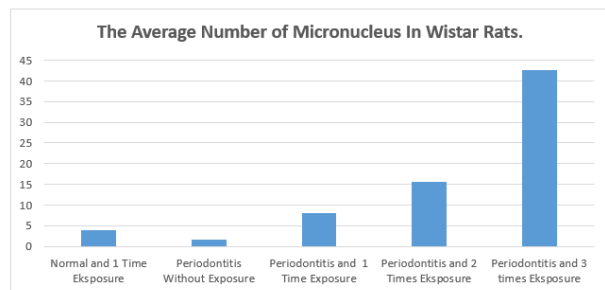


Figure 2. shows the average number of micronucleus in wistar rats. The highest average number of micronucleus was found in the periodontitis group with 3 times X-ray panoramic radiographic exposure.

The results and the average number of micronucleus were tabulated and analyzed using Saphiro-Wilk normality test. The results obtained  $p > 0.05$  which means that the data was normally distributed. Then, the data were analyzed using Levene's test of variance test and the result obtained  $p = 0.061$  ( $p > 0.05$ ) which means that the data was homogenously distributed.

One way ANNOVA test was subsequently conducted and was resulted in  $p = 0.000$  ( $p < 0.05$ ). This result depicted a significant difference between treatment. Data analysis continued with Post-Hoc Bonferroni to find out which groups made a significant difference.

Table 2. Results of Post-Hoc Bonferroni Statistical Test on Micronucleus Mikronukleus

	N and 1 Time Expos ure	P Witho ut Expos ure	P and 1 Time Expos ure	P and 2 Times Expos ure	P and 3 Times Expos ure
N and 1x Exposu re		1,000	1,000	0,016 *	0,000 *
P and Without Exposu re	1,000		0,429	0,004 *	0,000 *
P and 1x Exposu re	1,000	0,429		0,186	0,000 *
P and 2x Exposu re	0,016 *	0,004 *	0,186		0,000 *
P and 3x Exposu re	0,000 *	0,000 *	0,000 *		

Description:

N : Normal

P : Periodontitis

\* : There is significant difference ( $p < 0,05$ )

Table 2. shows that group that have a significant difference in the number of micronucleus were in the normal group and 1 time of X ray radiation exposure to periodontitis group with 2 and 3 times exposure, and in the periodontitis without exposure group to periodontitis group with 2 and 3 times exposure.

## DISCUSSION

Study of the effect of panoramic radiography on the number of micronucleus in wistar rats (*Rattus norvegicus*) with periodontitis showed that there were micronucleus in each treatment group.

The results of this study between the normal group with 1 time exposure and periodontitis group with 2 times exposure showed a significant difference. That is due to two factors that affect the formation of the micronucleus, that are the presence of periodontitis and doubled exposure.

In this study showed that the average number of micronucleus in the normal group with 1 time of exposure was 4 and in the periodontitis group with 2 times of exposure was 15.67 which means that periodontitis and 2 times repeated exposure could make a significant difference to the number of micronucleus. This is because the periodontitis can cause the formation of micronucleus. Reactive

Oxygen Species (ROS) can modify cellular proteins, lipids, and Deoxy Nucleic Acid (DNA) so that they change cell function.<sup>16</sup> In the case of periodontitis, the production of Reactive Oxygen Species (ROS), which is mostly derived from neutrophils, increases drastically and is involved in the destruction of periodontal tissue, as a result oxidative stress increases during periodontitis.<sup>1,17</sup>

Oxidative stress has spesific effects that can cause oxidative damage starting from the cellular level, tissue to the organs of the body and resulting in the emergence of various pathogenesis that leads to cancer through DNA damage that is characterized by the formation of micronucleus.<sup>16,1,18</sup> Repeated panoramic radiography radiation exposure also affects the number of micronucleus due to the severity of deterministic damage to the irradiated tissue or organ depending on the amount of radiation exposure received. The more exposure received, the greater damage that occurs. Provision of repeted radiation exposure will cause the tissue or organ does not have the opportunity to repair the damage so that it produces high effects due to the damage.<sup>19</sup>

The effects of panoramic radiographic radiation exposure can lead to the formation of micronuclei because X-rays produced by panoramic radiographic radiation exposure can cause damage to DNA that will result in chromosomal aberration.<sup>19</sup> Chromosomal aberration will cause the formation of micronuclei that originate from the eccentric chromosomes that are left behind during mitotic cells in the anaphase stage.<sup>20</sup> Micronucleus formed due to chromosomal aberration is an indication of mutagenic activity that can damage chromosomes.<sup>21</sup>

The existence of a micronucleus is a biomarker that provides information on cytogenetic damage in human tissue that is potentially affected by carcinogens.<sup>13</sup> Bastos-Aires (2013) mentioned that in a healthy periodontal tissue condition, the average number of micronuclei is 1.33 / 1000 cells.<sup>22</sup> The micronucleus test is considered an important strategy for monitoring oral precancerous lesions. According to Chatterjee (2009), this test is a simple and practical screening technique for prevention in individuals who are at risk of getting cancer.<sup>23</sup>

The above discussion concluded that the micronucleus can be formed due to panoramic radiographic radiation exposure with a periodontitis condition, so it must be minimized by increasing radiation protection efforts. The effort to protect against radiation is guided by three principles, among others, justifying, optimizing and limiting the administration of dosages.<sup>19</sup> BAPETEN 2019 stated that patients do not need dose limitation as the Dose Limit Value (NBD).

However, the dose received by the patient must remain justified and optimized so as to prevent unnecessary exposure or unintended radiation exposure to reduce the effects of risk on damage caused by radiation exposure.<sup>15,19</sup>

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