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The Effects of Panoramic Radiography on Gingival Crevicular Fluid Volume and Micronucleus in Wistar Rats (*Rattus Novergicus*) with Periodontitis

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Abstract: Panoramic radiography is a radiographic diagnostic tool that affects the Reactive Oxygen Species and causes oxidative stress, which has a role in DNA damage that occurs due to periodontitis or panoramic radiographic radiation exposure is characterized by the formation of micronuclei in gingival epithelial cells. Panoramic radiography exposure and periodontitis can increase the Gingival Crevicular Fluid (GCF) flow because of increased vascular permeability. The present study aims to determine the effect of panoramic radiography on the volume of GCF and micronuclei in Wistar rats with periodontitis. This study was true experimental with post-test only and control group design, used 40 male Wistar rats. The average number of GCF in the healthy rats' group that was not exposed to panoramic radiography was 0.114 μL, the periodontitis rats group that was not exposed, once, twice, and three times exposed to panoramic radiography were 0.246, 0.286, 0.294, and 0.374 μL, respectively. The result of the One-Way Annova test and Post Hoc Bonferroni test indicated that significant changes in the number of micronuclei were seen between the normal group with once exposure to the periodontitis group with two times and three times exposure and in the periodontitis group without exposure to the periodontitis group with two times and three times exposure. Panoramic radiograph X-ray radiation causes an increase in the volume of GCF and changes the number of micronuclei in Wistar rats with periodontitis.

Keywords: panoramic radiography, Gingival Crevicular Fluid, micronucleus, periodontiti.

全景射線照相術對患有牙周炎的威斯塔大鼠 (褐家鼠) 齦溝液量和微核的影響

摘要：全景射線照相是一種射線照相診斷工具，它影響活性氧種類並引起氧化應激，其在由於牙周炎或全景射線照相輻射暴露而發生的底部損傷中起作用，其特徵在於牙齦上皮細胞中微核的形成。由於血管通透性增加，全景射線照相曝光和牙周炎可增加齦溝液流量。本研究旨在確定全景放射照相術對患有牙周炎的威斯塔大鼠齦溝液和微核體積的影響。這項研究是真正的實驗，僅採用後測和對照組設計，使用了四十隻雄性威斯塔大鼠。未接觸全景片的健康大鼠組齦溝液平均為0.114微升，未接觸、1次、2次、3次全景片接觸的牙周炎大鼠組分別為0.246、0.286、0.294、分別為0.374微升。單向安諾瓦試驗和事後邦費羅尼試驗結果表明，正常組暴露1次，暴露2次和3次牙周炎組與未暴露牙周炎組的微核數發生顯著變化。牙周炎組2次、3次暴露。全景倫琴射線倫琴射線輻射導致齦溝液體積增加並改變患有牙周炎的威斯塔大鼠的微核數量。

关键词：全景射線照相術，齦沟液，微核，牙周炎。

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1. Introduction

Periodontitis is a periodontal disease that can cause the formation of Reactive Oxygen Species (ROS) [1, 2]. 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 [16]. This action increases 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 [1].

Oxidative stress also plays a role in causing DNA damage. One method to determine DNA damage is the micronucleus assay [1]. DNA damage is more common in individuals with periodontitis than individuals without periodontitis [3].

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. It can show a generalized horizontal bone loss from mild to moderate [4, 5]. Panoramic radiography in periodontitis patients is usually used twice, before and after treatment, to evaluate treatment results [5].

Panoramic radiography is one of the most common diagnostic tools in dentistry that uses X-ray radiation with a small wavelength and considerable energy to show a complete picture of the jaw [6, 7]. X-rays have a small wavelength and large energy included in one type of ionizing radiation that can ionize a material passed through [7-10].

The X-ray may cause a direct effect when its radiation molecule ionizes directly to the vital biological macromolecules such as DNA. In contrast, the indirect effect occurs when the radiation molecule ionizes the water molecule to form the free radicals and ions, which can combine and cause DNA damage [2, 11]. DNA damage is characterized by the formation of the micronucleus [10]. Sheikh et al. and Saputra et al. mentioned that micronucleus formed on gingival epithelial cells after panoramic radiography, and the largest number of micronucleus occurred on the 7th day after exposure to panoramic radiography [12, 13].

Micronucleus comes from chromosome fragments or all chromosomes left during the division of the nucleus (anaphase) and is found in cells with DNA damage [1, 14]. Micronucleus functions as a biomarker that provides information about cytogenic damage in human tissue potentially affected by carcinogens [15]. Shatingsih et al. mentioned that micronuclei could increase in epithelial cells in the gingival mucosa [16]. Increased micronucleus occurs due to migration of cells containing micronucleus in the basal stratum, then differentiated and keratinized towards a more superficial layer so that the micronucleus can be found in the mucosal epithelium [17].

X-ray radiation can cause an increase in vascular permeability due to ionizing radiation in endothelium cells [18]. An increase in vascular permeability due to panoramic radiographic radiation increased the GCF volume [16].

GCF is a transudate in a healthy sulcus and an exudate in an inflammatory state to describe the health status of periodontal tissue. Increased GCF flow in inflammatory conditions will make a dilution effect on bacterial products and a flushing effect to remove the bacteria and their products from the sulcus and bring the immune system components to the sulcus. Excessive GCF volume has an unfavorable effect, resulting in the formation of tartar (calculus) from the alkaline phosphatase presented in the GCF. Proteolytic enzymes in the GCF can also be dangerous for the gingival sulcus and other gingival tissue.

One of the periodontal diseases that can be found is periodontitis. In periodontitis, collagen damage may be present that can spread to the periodontal ligament and alveolar bone. The dominant neutrophils are found in the pocket epithelium and the periodontal pocket, and the dominant plasma cells are found in the connective tissue. Neutrophils will migrate from the gingival plexus through connective tissue outside the vascular and eventually go to the junctional epithelium through the basement membrane. This activity will certainly result in vascular changes in the gingiva, which causes an increase in GCF flow into the pocket [19-24].

2. Materials and Methods

The study of Panoramic Radiography effect on GCF was used 25 male Wistar rats aged 1.5-2 months and weighed 150-200 grams that were divided into 5 groups: a control group 1, including the mice without induction of periodontitis and exposure to panoramic radiographic radiation; group 2 as a control group 2, including the mice that were induced by periodontitis but not exposed to panoramic radiographic radiation; group 3 is a group of rats induced by periodontitis and given panoramic radiographic radiation exposure once; group 4 is a group of rats induced by periodontitis and exposed to radiographic panoramic radiation twice; group 5 was a group of rats induced by periodontitis and given panoramic radiographic radiation exposure 3 times.

In groups 2, 3, 4, and 5, periodontitis was induced with 0.03 ml inoculation of *P.gingivalis* $2x10^6$ CFU/ml. Bacteria were injected into the gingival sulcus of the right and left mandibular incisors every 3 days for 2 weeks to show clinical signs of periodontitis. After induction of periodontitis, groups 3, 4, and 5 were given panoramic radiography. The radiation dosage was calculated using a dosimeter, and an average radiation dose for 1 exposure was 0.05 mGy. The sample GCF of Wistar rats were then taken using filter paper strips. Before the sample was taken, first is isolating the area of the mandibular incisors with cotton rolls and slowly drying them with a water

syringe to avoid salivary contamination. The filter paper was carefully inserted into the gingival crevicular (sulcus) and left for 30 seconds. Measurement of the GCF volume was carried out using a 2% ninhydrin solution which was dripped on filter paper strips containing GCF samples until it turned purple. The three highest points of the purple area were measured using a digital sliding caliper and then averaged. GCF volume in mm^3 was calculated by multiplying the average purple area by the width and thickness of the filter paper.

The GCF volume in each group was summed and averaged. The results of this study were statistically tested using the SPSS 26 computer program. Data were tested for normality using the Shapiro-Wilk test and Levene's Test variance homogeneity test. In normally distributed and homogeneous data, it is followed by One-Way Anova parametric analysis with a confidence level of 95%. On data with a significant difference ($p < 0.05$), the Post Hoc Bonferroni test was proceeded to find out which groups have significant differences.

For the micronucleus study, the subjects were 15 male Wistar rats aged 1.5-2 months and weight 150-200 gram divided into 5 groups: normal and was given X-ray radiation exposure once; periodontitis without X-ray radiation exposure; periodontitis with 1, 2, and 3 times X-ray radiation exposure.

The first step was an adaptation of the Wistar rats for 7 days to obtain uniformity of the sample before treatment. Wistar rats that have been adapted were carried out by inducing Porphyromonas gingivalis for 2 weeks until they showed clinical signs of periodontitis. Then, 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 removed the gingival tissue of the Wistar rat to see the micronucleus. The micronucleus interpretation and calculation were performed using a light microscope with a magnification of 400× at five visual fields.

3. Results

Table 1 shows the effects of panoramic radiograph on GCF volume in Wistar rats with periodontitis.

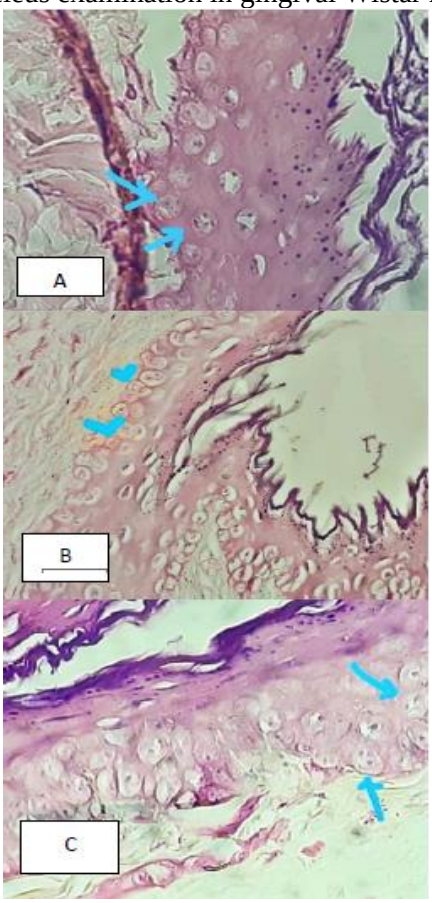
Table 1 GCF volume

Group	N	Mean ± SD Scoring (µL)
Control 1	5	0.114 ± 0.026
Control 2	5	0.246 ± 0.048
Induction of periodontitis with 1-time exposure	5	0.286 ± 0.073
Induction of periodontitis with 2 times exposure	5	0.294 ± 0.039
Induction of periodontitis with 3 times exposure	5	0.374 ± 0.047

It shows an increase between control group 1 and control group 2 that was induced with periodontitis showed an increase, as well as in groups 3, 4, and 5 that showed a gradual increase with the increased repetition of panoramic radiography radiation exposure, where the highest increase in GCF volume was in the group of periodontitis rats with 3 times exposure with the mean of GCF volume of 0.374 µL.

The Post Hoc Bonferroni analysis was done to find out the groups that have significant differences. The results showed that control group 1 showed significant differences with control group 2 and with the periodontitis induction group with 1, 2, and 3 times the exposure of panoramic radiography. As for control group 2, which is a periodontitis induction group without radiation exposure panoramic radiography, it was obtained that there was a significant difference with the periodontitis induction group with 3 times the exposure of panoramic radiography.

Below are the histopathological results of a micronucleus examination in gingival Wistar rats.



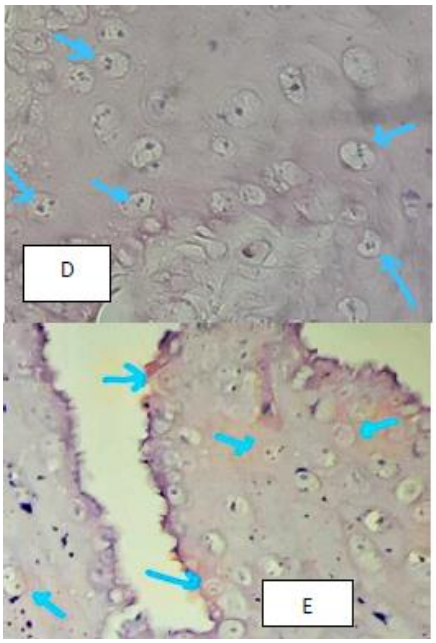


Fig. 1 Histopathology of micronucleus on the gingival epithelial cell of Wistar rats

Fig. 1A is the normal group with once exposure, Fig. 1B is periodontitis without exposure, Figs 1C, 1D, 1E are periodontitis groups 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 400-times magnification light microscope with five 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 below.

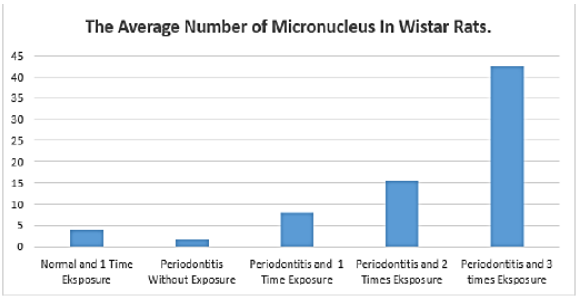


Fig. 2 The average number of micronucleus in Wistar rats

The highest average number of micronucleus was found in the periodontitis group with 3 times X-ray panoramic radiographic exposure. Post-Hoc Bonferroni analysis showed a significant difference in the number of micronuclei 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.

4. Discussion

GCF flow may increase during an inflammatory state in the form of periodontal diseases, such as periodontitis. The amount of GCF will be greater along with the severity of the periodontal disease. It is shown from the results of this study that there are significant differences between the control group and the group induced with periodontitis. In addition, in a study conducted by Attar et al., the average GCF volume will increase with the increase of probing depth which causes expansion in the surface area of the crevicular gingival [25]. In addition, the increase in gingival inflammation severity will result in the increased permeability of the gingival epithelium so that GCF volume will rise [23].

Moreover, GCF flow can increase due to panoramic radiography exposure. The results in this study indicate that there are significant differences between the control group and the periodontitis group exposed to panoramic radiographic radiation. These are in line with the research conducted by Zuelkevin that there is an increase in GCF volume in patients exposed to panoramic radiographic radiation [37]. The effect of panoramic radiographic radiation on increasing the number of GCF itself is related to increased vascular permeability due to ionizing radiation to vascular endothelial cells [16, 18] X-rays, which are commonly used in radiodiagnostics, can ionize cells through direct effects by directly ionizing macromolecules biologics such as DNA, and through indirect effects by ionizing water molecules around DNA so that it will produce ions and free radicals which together will cause damage to biological macromolecules such as DNA. As a result of biological damage to this DNA, it will cause damage to the cells. Endothelial cells, which are cells in blood vessels, can have lethal or sublethal damage due to radiation ionization, one of which is the increased permeability of blood vessels. An increase in vascular permeability will cause an increase in GCF flow. Plasma fluid will circulate from the blood vessels to the gingival crevicular region through the junctional epithelium [7, 11, 18, 26].

Based on the results of this study, addition in the frequency of panoramic radiography repetition, which causes the received dose to increase, will escalate the effects on the body, especially in increasing the GCF volume. The value of diagnostic reference levels (DRL) values for panoramic radiography is 0.66-4.2 mGy [7, 27, 28].

The periodontitis group without exposure had a significant difference from the periodontitis group exposed to 3 times panoramic radiographic radiation who received an average dose of 0.15 mGy, where this dose was indeed far from the DRL value that had been proven previously. A significant difference in 3 times exposure shows that increasing the amount of dose will also increase the effects caused. A higher dose rate can also influence the effects of the radiographic procedure. At a high dosage rate, i.e., the repetition procedure of radiography will make the tissue having

no opportunity to repair, thus the risk that may be caused by tissue damage will increase even higher. A higher dose rate will have a greater effect than larger amounts but with a lower rate of dose [7].

The increased GCF volume has a negative effect that can trigger calculus formation due to the presence of alkaline phosphatase (ALP) enzyme in the GCF and proteolytic enzymes, which can increase bone destruction activity [20]. The formation of calculus induced by ALP will go through a process where ALP will release orthophosphate that can cause an increase in the concentration of orthophosphate, which will react with calcium ions and lead to the formation of calcium apatite crystal deposits to form calculus. The presence of proteolytic enzymes that are released in the form of matrix metalloproteinases (MMPs), in the type of MMP-8, will break down the structure of proteins in connective tissue in the gingiva initiate collagen destruction. It will lead to more severe bone destruction [23, 29].

Based on this study, the GCF volume in the periodontitis group that was given 1 time of exposure was 0.288 μL, the group with 2 times exposure was 0.294 μL, and the group with 3 times exposure was 0.372 μL. The normal GCF volume in humans in the anterior teeth region is ranged from 0.24 to 0.43 μL [11]. If it is compared with the GCF volume of Wistar rats at 1, 2, and 3 times the exposure of panoramic radiography, the volume was still within the normal limits. However, the range of normal GCF in Wistar rats can also differ from humans. In addition, this study still cannot be proven whether an increase in the GCF volume in once, twice and three times repeated exposures of panoramic radiographic could trigger the negative effects of the increase in GCF, which generates calculus formation induced by alkaline phosphatase enzyme from GCF and increased bone destruction due to the presence of proteolytic enzymes in GCF.

Based on the results of this study, it can be concluded that there is an increase in the GCF volume on Wistar rats with periodontitis due to X-ray radiation of panoramic radiography. This research is restricted to just measuring the volume of GCF, so further research on how large the cellular component in the GCF is due to the effects of X-ray radiation exposure which can cause a negative effect of the GCF, which can potentially aggravate the periodontitis is needed.

The study of the effect of panoramic radiography on the number of micronucleus in Wistar rats (*Rattus novergicus*) with periodontitis showed that there was micronucleus in each treatment group.

The study results showed a significant difference between the normal group once exposed and the periodontitis group twice exposed. That is due to two factors that affect the formation of the micronucleus, which are the presence of periodontitis and doubled exposure.

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 micronuclei. This is because periodontitis can cause the formation of a micronucleus. Reactive Oxygen Species (ROS) can modify cellular proteins, lipids, and Deoxy Nucleic Acid (DNA) so that they change cell function [30]. In the case of periodontitis, the production of 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 [1, 31].

Oxidative stress has specific 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 characterized by the formation of micronucleus [1, 30, and 32]. 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 repeated radiation exposure will cause the tissue or organ not to have the opportunity to repair the damage so that it produces high effects due to the damage [7].

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 [7] Chromosomal aberration will cause the formation of micronuclei that originate from the abnormal chromosomes left behind during mitotic cells in the anaphase stage [33]. Micronucleus formed due to chromosomal aberration is an indication of mutagenic activity that can damage chromosomes [34].

A micronucleus is a biomarker that provides information on cytogenic damage in human tissue potentially affected by carcinogens [13]. Bastos-Aires mentioned that the average number of micronuclei in a healthy periodontal tissue condition is 1.33/1000 cells [35]. The micronucleus test is considered an important strategy for monitoring oral precancerous lesions. According to Chatterjee, this test is a simple and practical screening technique for preventing individuals at risk of getting cancer [36].

The above discussion concluded that the micronucleus could be formed due to panoramic radiographic radiation exposure with a periodontitis condition, which must be minimized by increasing radiation protection efforts. The effort to protect against radiation is guided by three principles: justifying, optimizing, and limiting the administration

of dosages [19]. The dose received by the patient must remain justified and optimized to prevent unnecessary exposure or unintended radiation exposure to reduce the effects of risk on damage caused by radiation exposure [7, 27].

5. Conclusion

Panoramic radiograph X-ray radiation causes an increase in the volume of GCF and changes the number of micronuclei in Wistar rats with periodontitis. GCF flow may increase during an inflammatory state in the form of periodontal diseases, such as periodontitis. The amount of GCF will be greater along with the severity of the periodontal disease. It is shown from the results of this study that there are significant differences between the control group and the group induced with periodontitis. In addition, in a study conducted by Attar et al., the average GCF volume will increase with the increase of probing depth which causes expansion in the surface area of the crevicular gingival [25]. In addition, the increase in gingival inflammation severity will result in the increased permeability of the gingival epithelium so that GCF volume will rise [23]. This is in line with the research conducted by Zuelkevin that there is an increase in GCF volume in patients exposed to panoramic radiographic radiation [37]. The effect of panoramic radiographic radiation on increasing the number of GCF itself is related to increased vascular permeability due to ionizing radiation to vascular endothelial cells. The increased GCF volume has a negative effect that can trigger the formation of calculus due to the presence of alkaline phosphatase (ALP) enzyme in the GCF and proteolytic enzymes, which can increase bone destruction activity [20]. The formation of calculus induced by ALP will go through a process where ALP will release orthophosphate that can cause an increase in the concentration of orthophosphate, which will react with calcium ions and lead to the formation of calcium apatite crystal deposits to form calculus. The presence of proteolytic enzymes that are released in the form of matrix metalloproteinases (MMPs), in the type of MMP-8, will break down the structure of proteins in connective tissue in the gingiva initiate collagen destruction. It will lead to more severe bone destruction [23, 29].

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