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THE EFFECTS OF PANORAMIC RADIOGRAPHY ON GINGIVAL CREVICULAR FLUID VOLUME IN GINGIVITIS

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ABSTRACT

Background: *Gingival crevicular fluid (GCF) is a biological fluid derived from the gingival sulcus and can be elevated in the inflammatory state of periodontal tissue, such as gingivitis. In previous studies, the number of GCF could also increase after panoramic radiographic exposure. Increase in GCF due to panoramic radiography is a sign of cell damage. Objective:* To analyze the effects of panoramic radiography on the volume of GCF in wistar rats with gingivitis. **Method:** This type of research was true experimental with post test only and control group design. The sampling technique used was simple random sampling. A total of 25 wistar rats were classified into two control groups without exposure (state without gingivitis and gingivitis) and three groups treatment of exposure (state of gingivitis with 1 time, 2 times, and 3 times the exposure). GCF sampling using filter paper was carried out 10 minutes after panoramic radiographic exposure. The filter paper was stained by 2% ninhydrin solution, after that the GCF volume was calculated. **Results:** There was a significant difference in the number of GCF ($p < 0.05$) in the group without gingivitis and exposure compared to all other groups, the gingivitis group without exposure compared to the gingivitis group with 3 times exposure, and the gingivitis group with 1 time exposure compared to the gingivitis group 3 times with exposure. **Conclusion:** Panoramic radiography can cause an increase in the volume of GCF in wistar rats with gingivitis.

Keywords: GCF, gingivitis, panoramic radiography

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INTRODUCTION

Periodontal disease is one of the dental and oral diseases that are often found in Indonesia. Based on data from Riskesdas 2018, the prevalence of dental and oral health problems in Indonesia is 57.6% and the prevalence of periodontal disease is 73.1-75%.¹ Periodontal disease that is often found is gingivitis. Gingivitis is an inflammatory response that occurs at the gingival margin by the presence of plaque accumulation on the tooth surface.²

Gingivitis will increase gingival crevicular fluid (GCF) flow.³ GCF is a transudate that flows continuously from the crevicular gingiva with a low flow rate in healthy individuals.⁴ During inflammation, the flow of GCF increases and its composition begins to resemble an inflammatory exudate.³ GCF has a protective effect, but also has a negative effect on periodontal tissue through increased levels of alkaline phosphatase which triggers calculus formation and triggers the activity of proteolytic enzymes that are harmful to the gingival sulcus and other gingival tissue.⁵ Based on this, untreated gingivitis can progress to

periodontitis, so an appropriate examination is needed to assist in planning treatment so that gingivitis does not progress to periodontitis.⁶

Gingivitis is a periodontal disease that can be detected using clinical examination and radiographic examination. Clinical examination can provide information about soft tissue conditions such as redness, swelling, bleeding on probing and false pocket.⁷ Radiographic examination can provide a picture of damage of the alveolar crest for several cases of severe gingivitis such as Necrotizing Ulcerative Gingivitis (NUG), Acute Ulcerative Gingivitis (AUG), and Generalized marginal gingivitis. One radiographic technique that can be used for such cases is panoramic radiography. This panoramic radiography can help in determining the treatment plan to be performed.^{8,9}

Panoramic radiography or pantomography is a radiographic technique that produces an image with a broad structure in the maxillomandibular area and its supporting tissue structure. The dose used in panoramic radiographic exposure is relatively low, but it can still potentially cause biological changes in living tissue.¹⁰

These changes occur through ionization reactions and the formation of free radicals that can cause cell damage.^{10,11}

Each cell has a different response to radiation. One of the cells with relatively moderate radiosensitivity is blood vessel endothelial cells.¹⁰ In blood vessel walls, the most radiosensitive cell compared to other mesenchyme cells is endothelial cells. Damage to endothelial cells as an inflammatory response to radiation exposure results in increased vascular permeability.¹² Increased permeability of blood vessels will increase leakage of fluid coming out of blood vessels and facilitate the journey of defense cells from blood vessels to tissues. Increased fluid leakage can also increase hydrostatic pressure on local microcirculation, increase of osmotic gradient, and plasma fluid flow across the basement membrane to the region gingival crevicular through junctional epithelium, resulting in an increase in GCF.^{13,14}

Radiation with high dose level has a greater risk of damage than exposures with low dose levels. When organisms are exposed to low dose level, there is a greater chance of repairing the damage, resulting in less damage. In fact, the greater effect of radiation on body cells can occur due to the repetition of panoramic radiographs that are mediated by technical errors.^{10,15}

So far, research on GCF as a marker of the state of periodontal tissue has been carried out, but there is no link between gingivitis and panoramic radiographic exposure through GCF counts. Based on the description above, it is necessary to conduct a research to determine the effect of panoramic radiography on the number of GCF in the wistar rats (*Rattus norvegicus*) with gingivitis. Researchers want to know the effect of panoramic radiography on the number of GCF in wistar rats using exposure doses with consideration of the possibility of technical errors that can occur in the field 1 time, 2 times and 3 times.¹⁵

MATERIALS AND METHODS

In this study, an ethics feasibility test was conducted and published by the Faculty of Dentistry, Lambung Mangkurat University No.100 / KEPKG-FKGULM / EC / II / 2020. This type of research was a true experimental with a post test only and control group design and used simple random sampling. The sample used was 25 male wistar rats with healthy condition, weighing 250-300 grams and aged 3-4 months. Samples were classified into five groups: two control groups (C) and three treatment groups (T). Group C1 was a state without gingivitis and without exposure, Group C2 stated of gingivitis without exposure, Group T1 stated of gingivitis with 1 time panoramic radiographic exposure, Group T2 stated of gingivitis with 2 times panoramic radiographic exposure, and Group T3 stated of gingivitis with 3 times panoramic radiographic exposure. Before starting the study, the sample was adapted for 7 days first.

The making of the *Porphyromonas gingivalis* ATCC 33277 culture was carried out through a process, those were: (1) mixing 3.7 grams of BHI-A with 100 ml of sterile distilled water and then heating using an electric stove until homogeneous; (2) covering with a cotton swab and sterilize in an autoclave at 121° C for 15 minutes; (3) add 10µl of vitamin K, 50µl of hemin, and 500µl of yeast extract and then homogenized; and (4) put it in an incubator for 24 hours for the sterilization test.¹⁶

The making of BHI-B media was carried out through a process, those were: (1) mixing 3.7 grams of BHI-B with 10 ml of sterile aquades in an erlenmeyer tube then heat using an electric stove until homogeneous, (2) cover using cotton and sterilize in an autoclave on temperature 121°C for 15 minutes, (3) add 1µl of vitamin K, 5µl of hemin, and 50µl of yeast extract and then homogenized; and (4) put it in an incubator for 24 hours for the sterilization test. Preparation of *P. gingivalis* suspension carried out by mixing 1 ose of *P. gingivalis* with 2 ml of BHI-B media in a test tube until it was equivalent to the turbidity standard of Mc Farland 1 which is 0.10 BaCl₂ and 0.90 H₂SO₄.¹⁶

The gingivitis induction procedure was performed by induction of 0.02 ml of *P. gingivalis* with a concentration of 3x10⁸ CFU using a disposable syringe on the mandibular incisor gingival sulcus for 2 days. The clinical manifestation of gingivitis was seen on day 3, such as redness and gingival swelling.¹⁶

Panoramic radiographic exposure procedures were performed in all treatment groups. Prior to the radiation exposure, wistar rats were given anesthesia with ketamine.¹⁷ Anesthetized wistar rats were placed at a height parallel to the chinrest panoramic radiography machine so that it can resemble the patient position during the panoramic radiographic procedure. The exposure procedure was carried out using the Villa Rotograph EVO panoramic radiography machine with an electric voltage of 70 kV, an electric current of 60 mA, and a dose of 0.05 mGy for one exposure. The exposures were carried out 1 time, 2 times, and 3 times with a repetition interval of 1 minute.

The GCF sampling procedure was performed 10 minutes after panoramic radiography exposure. GCF was taken when the wistar rat was under anesthesia. In the experimental area that is around the mandibular incisors, it was cleaned using cotton rolls to control saliva. Paper filter strips were carefully inserted into the gingival sulcus using tweezers and excavators to help insert the paper filter strips until there was a moderate resistance to the paper filter strips in the gingival sulcus and left for 30 seconds.¹⁸

Calculating the amount of GCF was done using a 2% ninhydrin solution which was dripped on a filter paper containing GCF samples, then waited for the filter paper for color changing. After that, GCF samples in the filter paper produced a purple area. The three highest points of the purple color area were measured using a

digital sliding caliper, then the average was counted. GCF volume in mm³ was calculated by multiplying the average of purple area by the width and thickness of the filter paper, then converting to μL.¹⁸

RESULTS

The average value of the number of GCF obtained in this study can be seen in the following Table 1.

Table 1. Average GCF number of wistar rats in all control and treatment groups

Group	N	Mean ± SD Scoring (μL)
C1	5	0,154627±0,0214122
C2	5	0,265833±0,0212844
T1	5	0,268800±0,0525053
T2	5	0,308867±0,0437808
T3	5	0,391233±0,0757592

Note:

- C1 : No gingivitis and no exposure
- C2 : Gingivitis without exposure
- T1 : Gingivitis with 1 time exposure
- T2 : Gingivitis with 2 times exposure
- T3 : Gingivitis with 3 times exposure

Table 1 shows an increase in GCF after wistar rats developed gingivitis and received panoramic radiographic radiation. The greater the frequency of panoramic radiographic x-ray radiation exposure, the greater the amount of GCF in wistar rats with gingivitis. This shows that the more the frequency of panoramic radiographic repetition, the greater the effect on the body.

Data on the number of GCFs that have been obtained in all control and treatment groups were tested by Saphiro-Wilk normality test and each group had a value of p>0.05, which means that the data were normally distributed. After that, the data was carried out homogeneity tests using Levene's test and obtained a significance value of 0.055 (p≥0.05) which means homogeneous distributed data. The data was then performed One-way ANOVA parametric analysis with a confidence level of 95% and obtained a significance value of 0,000 (p<0.05) which showed a significant difference between treatments. After that, Post Hoc Bonferroni test was performed to find out which groups have significant differences.

Table 2. Statistical Test Results Post Hoc Bonferroni

	C1	C2	T1	T2	T3
C1		0,014*	0,011*	0,001*	0,000*
C2	0,014*		1,000	1,000	0,005*
T1	0,011*	1,000		1,000	0,006*
T2	0,001*	1,000	1,000		0,127
T3	0,000*	0,005*	0,006*	0,127	

Note:

* = There is a significant difference (p<0,05)

Based on the Post Hoc Bonferroni test, the number of GCF in the C1 group compared to all other groups

showed a value of p<0.05, which means there were significant differences. The C2 group compared to the T3 showed a value of p<0.05 which means that there were significant differences. The T1 and the T2 group to the C2 showed value of p>0.05, which means there was no significant difference. The T3 group compared to the T1 showed value of p>0.05, which means that there was a significant difference.

DISCUSSION

Based on the result of this study, the condition without gingivitis and exposure produced GCF amount of 0.154627 μL, gingivitis without exposure produced 0.264833 μL, gingivitis with 1 time exposure produced 0.268800 μL, gingivitis with 2 times exposure produced 0.308867 μL, and gingivitis with 3 times exposure produced 0.391233 μL. This shows that the condition of gingivitis with 1 time, 2 times, and 3 times of exposures was proven to have an effect on the number of GCF, which resulted in an increase in the number of GCF.

The condition without gingivitis and exposure results in a lower amount of GCF than gingivitis without exposure. This is consistent with previous research conducted by Akman et al (2017) which stated that a healthy periodontal tissue condition can produce GCF amount of 0.37 μL, whereas inflammatory conditions results in an increase of GCF amount to as many as 0.50 μL.¹⁹ This is caused by the condition of gingivitis which can lead to an increase in the rate of GCF flow to the area where microbial penetration occurs.¹⁴

When there is microbial penetration in periodontal tissue, innate immune response, that work quickly, are non-specific and become the first line of defense against microbial penetration. This immune response involves the epithelial barrier, complement, cytokines, neutrophils, and mast cells. The epithelial barrier has a role in initiating and spreading the inflammatory response through the release of cytokines after stimulation of bacterial products. Bacterial products such as endotoxins can activate a complement containing 20 serum glycoproteins in blood and tissue fluid. Supplements that are activated together with cytokines (IL-1, IL-6, IL-8, IL-10, TNF-α, PGE₂) will trigger pro-inflammatory activity. The pro-inflammatory activity triggers mast cells to activate and degranulate. When mast cells degranulate, there will be an increase in permeability of blood vessels through dilated capillary blood vessels which helps the migration of inflammatory mediators from the blood into the tissues.^{2,20} Migration of inflammatory mediators can also facilitate the entry of macromolecules into the fluid, so that the production of fluid from capillaries is greater than the absorption of gingival lymphatics and results in an increase in GCF.^{5,13}

The state of gingivitis with 1 time exposure results in a higher amount of GCF than gingivitis without exposure. This is consistent with previous research

conducted by Shantiningsih and Diba (2018) which stated that prior to X-ray radiography, it is only 0.14 μL , whereas the number of GCF after X-ray radiography radiography has been increased to 0.21 μL .²¹ A panoramic radiograph that produced X-ray radiation can cause damage to cells through two pathways mechanism, which is the directly and indirectly mechanism. The mechanism of direct cell damage occurs through X-ray ionization that directly hits the cell and causes DNA damage, while indirect damage occurs through the formation of free radicals which have a very damaging effect on DNA.¹⁰

Cell damage due to ionizing radiation can occur in endothelial cells because it is the most radiosensitive cell compared to other mesenchymal cells in blood vessels. Damage to endothelial cells can greatly change the complex physiology of endothelium. One of the changes that occur in endothelial cells is an increase in the permeability of blood vessels.^{12,22} Increased vascular permeability will increase leakage of fluid coming out of blood vessels and facilitate the journey of defense cells from blood vessels to tissues. Increased fluid leakage can also increase hydrostatic pressure on local microcirculation, increase osmotic gradient, and plasma fluid flow across the basement membrane to the region gingival crevicular through junctional epithelium, resulting in an increase in GCF.^{13,14,21}

The state of gingivitis with 2 times exposure results in a higher amount of GCF than the gingivitis with 1 time of exposure. That is because the radiation dose produced at 2 times panoramic radiographic exposure is 0.1 mGy. The dose is greater than a single panoramic radiographic exposure dose of 0.05 mGy. However, the dose is not much different. When organisms are exposed to low dose level, there is a greater chance of repairing the damage, resulting in less damage.¹⁰

The state of gingivitis with 3 times exposure had the highest GCF number. This happens because the radiation dose produced at 3 times panoramic radiographic exposure which is 0.15 mGy. That is greater than the dose of 2 times of exposure and 1 time of exposure. This is also in line with research by Puspitaningrum et al (2018) which said that the more X-ray exposure is carried out, it will result in an increase of the effects of damage.²³ Based on research by Tsapaki (2017), it was found that Diagnostic Reference Levels (DRLs) or acceptable dose limits for patients for panoramic radiographic exposure are 0.66 - 4.2 mGy.²⁴ Based on this study, the dose of 3 times the exposure is still below the DRLs threshold, but according to White and Pharoah (2014) it is stated that the damage can also be influenced by the dose level. At high dosage levels, there is a risk of higher damage than exposure to low dose levels.¹⁰

An increase in the amount of GCF can result in an increase in alkaline phosphatase levels which triggers calculus formation.⁵ Increased levels of alkaline

phosphatase can release inorganic orthophosphate from organic phosphate and trigger an increase in orthophosphate concentration locally. Orthophosphate can trigger an increase in mineralization through its interaction with calcium ions and leads to precipitation of insoluble calcium apatite crystals, leading to calculus formation.²⁵ However, in this study, it has not been proven whether the increase in GCF amount due to repeated panoramic radiographic X-ray radiation exposure 1 time, 2 times, and 3 times can trigger calculus formation induced by the alkaline phosphatase enzyme in GCF.

The increasing number of GCF as a sign of cell damage due to panoramic radiography can be minimized by further increasing the radioprotective effort of the patient. Increased radioprotective effort can be done by minimizing the possibility of technical errors that can occur when doing panoramic radiograph, thereby preventing the repetition of panoramic radiographic exposure and preventing the increase in dose absorbed by the patient.^{10,15}

Based on the explanation above, it is concluded that panoramic radiography with the frequency of repeated exposures of 1, 2 times, and 3 times in wistar rats with gingivitis was proven to have an effect on the number of GCF, namely an increase in the number of GCF. The more the frequency of repeated panoramic radiographic exposures in gingivitis, the higher the amount of GCF produced.

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