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Panoramic Radiography Effects on Gingival Crevicular Fluid Volume in *Rattus Novergicus* with Gingivitis and Periodontitis

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Abstract: Panoramic radiography exposure can increase the Gingival Crevicular Fluid (GCF) flow because of increased vascular permeability, and then GCF flow increases risk in periodontitis and gingivitis conditions. This study aims to analyze the effects of panoramic radiography on the volume of GCF in Wistar rats with gingivitis and periodontitis. This study was true experimental with a post-test-only control group design. The subject was 25 Wistar rats with a gingivitis group comprised of 5 Wistars from each group in which the GCF samples of each group were collected from. For the periodontitis, the state was used 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).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. The average number of GCF in healthy rats group without periodontitis that was not exposed to panoramic radiography was 0.114 µL, the periodontitis rats group that was not exposed to panoramic radiography was 0.246 µL, the periodontitis rats group that was exposed 1 time was 0.286 µL, the group of periodontitis rats exposed 2 times was 0.294 µL, and periodontitis group of rats exposed 3 times was 0.374 µL. Panoramic radiography can cause an increase in the volume of GCF in Wistar rats with gingivitis and periodontitis.

Keywords: panoramic radiography, Gingival Crevicular Fluid, gingivitis, periodontitis.

全景射線照相術對患有牙齦炎和牙周炎的褐家鼠齦溝液量的影響

摘要：由於血管通透性增加，全景射線照相曝光可以增加齦溝液流量，然後齦溝液流量會增加牙周炎和牙齦炎的風險。本研究旨在分析全景攝影對牙齦炎和牙周炎威斯塔大鼠齦溝液體積的影響。這項研究是真正的實驗，僅採用後測試控制組設計。受試者是25只患有牙齦炎的威斯塔大鼠，由每組的5只威斯塔組成，其中收集了每組的齦溝液樣品。對於牙周炎，使用狀態將25只威斯塔大鼠分為2個未暴露對照組(無牙齦炎和牙齦炎狀態)和3組暴露治療組(牙齦炎1次、2次和3次暴露)。牙齦炎未暴露組與其他所有組相比，牙齦炎未暴露組與牙齦炎暴露3次組相比，牙齦炎暴露組與牙齦炎暴露3次組相比有顯著差異（磷<0.05），牙齦炎暴露

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組與1時間暴露相比牙齦炎組暴露3次。未暴露於全景片的無牙周炎健康大鼠組的平均齦溝液數為0.114微升，未暴露於全景片的牙周炎大鼠組為0.246微升，暴露1次的牙周炎大鼠組為0.286微升，牙周炎大鼠暴露2次組為0.294微升，牙周炎大鼠暴露3次組為0.374微升。全景射線照相可導致患有牙齦炎和牙周炎的威斯塔大鼠的齦溝液體積增加。

关键词：全景射線照相，齦溝液，牙齦炎，牙周炎。

1. Introduction

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 [1-6].

Gingivitis is an inflammatory response that occurs at the gingival margin by plaque accumulation on the tooth surface [7]. Gingivitis will increase gingival crevicular fluid (GCF) flow [8]. GCF is a transudate that flows continuously from the crevicular gingiva with a low flow rate in healthy individuals [9]. During inflammation, the flow of GCF increases, and its composition begin to resemble an inflammatory exudate [8]. GCF has a protective effect but harms periodontal tissue through increased levels of alkaline phosphatase, which triggers calculus formation and activates proteolytic enzymes that are harmful to the gingival sulcus and other gingival tissue [10].

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 [11]. These changes occur through ionization reactions and the formation of free radicals that can cause cell damage [11, 12].

Each cell has a different response to radiation. One of the cells with relatively moderate radiosensitivity is blood vessel endothelial cells [11]. 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 increases vascular permeability [13]. Increased permeability of blood vessels will increase fluid leakage 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, an increase of osmotic gradient, and plasma fluid flow across the basement membrane to the region gingival crevicular through the junctional epithelium, increasing GCF [5, 14].

Radiation with high dose levels has a greater risk of damage than exposures with low dose levels. When organisms are exposed to low dose levels, there is a greater chance of repairing the damage, resulting in less damage. The greater effect of radiation on body cells can occur due to the repetition of panoramic radiographs mediated by technical errors [11, 15].

So far, research on GCF as a marker of the state of periodontal tissue has been carried out but without a link between gingivitis and panoramic radiographic exposure through GCF counts. Based on the description above, it is necessary to research to determine the effect of panoramic radiography on the number of GCF in the Wistar rats (*Rattusnovergicus*) with gingivitis and periodontitis.

2. Materials and Methods

The study of panoramic radiography effect on GCF volume in Wistar rats (*Rattusnovergicus*) with gingivitis was used 25 male Wistar rats with a 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 without gingivitis, and Group C2 consisted of gingivitis without exposure, Group T1 consisted of gingivitis with 1-time panoramic radiographic exposure, Group T2 consisted of gingivitis with 2 times panoramic radiographic exposure, Group T3 consisted of gingivitis with 3 times panoramic radiographic exposure. Before starting the study, the sample was adapted for 7 days first.

The *Porphyromonas gingivalis* ATCC 33277 culture was made through a process of (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 10 µl of vitamin K, 50µl of hemin, and 500 µl of yeast extract and then homogenized; and (4) put it in an incubator for 24 hours for the sterilization test [16].

Making BHI-B media was through (1) mixing 3.7 grams of BHI-B with 10 ml of sterile equates in an Erlenmeyer tube then heat using an electric stove until homogeneous, (2) cover using cotton and sterilize in an autoclave on temperature 121ºC 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 was carried out by mixing one dose 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 $BaCl_2$ and 0.90 H_2SO_4 [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 two 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. Before 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 to resemble the patient's 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].

The amount of GCF was calculated 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, and then the average was counted. GCF volume in mm3 was calculated by multiplying the average of the purple area by the width and thickness of the filter paper, then converting it to μL [18].

For the Wistar rat with periodontitis was used 25 male Wistar rats aged 1.5-2 months and weighed 150-200 grams were divided into 5 groups. The treatment groups consisted of group 1 as 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 1 time, group 4 is a group of rats induced by periodontitis and exposed to radiographic panoramic radiation 2 times, and 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* 2x106 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. Using the Villa Rotograph EVO panoramic radiograph unit, panoramic radiographic exposure was 1, 2, and 3 times.
A radiation dosage was calculated using a dosimeter during the radiation exposure, and an average radiation dose for one 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 mm3 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.

3. Results

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

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

Group	N	Mean
C1: No gingivitis and no exposure	5	0.154627 ± 0.0214122
C2: Gingivitis without exposure	5	0.265833 ± 0.0212844
T1: Gingivitis with 1-time exposure	5	0.268800 ± 0.0525053
T2: Gingivitis with 2 times exposure	5	0.308867 ± 0.0437808
T3: Gingivitis with 3 times exposure	5	0.391233 ± 0.0757592

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 GCF in Wistar rats with gingivitis, so, the more panoramic radiographic repetition frequency, the greater the impact on the body.

Data on the number of GCFs obtained in all control and treatment groups were tested by the Shapiro-Wilk normality test. 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 ($p \geq 0.05$) which means homogeneously 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, a Post Hoc Bonferroni test was performed to find out which groups have significant differences.

The number of GCF in the C1 group compared to all other groups showed $p<0.05$, which means there were significant differences. C2 group compared to T3 showed $p<0.05$, which means significant differences. T1 and T2 groups compared to C2 showed the value of $p>0.05$, which means there was no significant difference. T3 group compared to T1 showed a value of $p>0.05$, which means a significant difference.

The effect of panoramic radiograph on GCF volume in Wistar rats with periodontitis is an increase in the average GCF volume after treatment that can be seen in Table 2.

Table 2 Mean of GCF volume in Wistar rats

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

Based on Table 2, the mean of GCF volume 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.

Data were tested with the Shapiro-Wilk test for its normality resulting in $p>0.05$, which shows that the data were normally distributed. Data were tested with Levene's homogeneity test, resulting in $p = 0.097$ ($p> 0.05$), which shows that the data were homogeneous. Data normally distributed and homogeneous were followed by analysis using One-way ANOVA parametric analysis with a confidence level of 95% resulting in $p = 0.000$ ($p <0.05$), which indicates a significant difference between treatments. The data analysis was continued with Post Hoc Bonferroni analysis to find groups with 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 of panoramic radiography exposure.

4. 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 affect the number of GCF, which increased the number of GCF.

The condition without gingivitis and exposure resulted in a lower amount of GCF than gingivitis has without exposure that is consistent with previous research by Akman et al., which stated that a healthy periodontal tissue condition could produce a GCF amount of 0.37 µL; in contrast, inflammatory conditions result 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 responses 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 by releasing 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 activate together with cytokines (IL-1, IL-6, IL-8, IL-10, TNF-α, PGE2) will trigger proinflammatory activity. The proinflammatory 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 [19, 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 [10, 14].

The state of gingivitis with 1-time exposure results in a higher amount of GCF than gingivitis without exposure that is consistent with previous research conducted by Shantiningsih and Diba that stated that before X-ray radiography, it is only 0.14 µL; in contrast, the number of GCF after X-ray radiography has been increased to 0.21 µL [21].

A panoramic radiograph that produces 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; in contrast, indirect damage occurs through the formation of free radicals, which have a very damaging effect on DNA [11].

Cell damage due to ionizing radiation can occur in endothelial cells because it is the most radiosensitive cell compared to other mesenchymal blood vessels. Damage to endothelial cells can greatly change the complex physiology of endothelium. One of the changes in endothelial cells is an increase in the permeability of blood vessels [13, 22]. Increased vascular permeability will increase fluid leakage 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 the junctional epithelium, increasing GCF [5, 14, and 23].

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 [11].

The state of gingivitis with 3 times exposure had the highest GCF number because the radiation dose produced at 3 time's panoramic radiographic exposure, which is 0.15 mGy that is greater than the dose of 2 times 1 time of exposure. This picture is also in line with research by Puspitaningrum et al., which said that the more X-ray exposure is carried out, it will increase the effects of damage [25]. Based on research by Tsapaki, 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, 3 times exposure is still below the DRLs threshold, but according to White and Pharoah, the dose level can also influence the damage; in contrast, at high dosage levels, there is a risk of greater damage than exposure to low dose levels [11].

An increase in the amount of GCF can increase alkaline phosphatase levels, which triggers calculus formation [5]. Increased alkaline phosphatase levels 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 the precipitation of insoluble calcium apatite crystals, leading to calculus formation [25]. However, this study proved that the increase in GCF amount due to repeated panoramic radiographic X-ray radiation exposure 1 time, 2 times, and 3 times could 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 made by minimizing the possibility of technical errors when doing panoramic radiographs, thereby preventing the repetition of panoramic radiographic exposure and preventing the increase in dose absorbed by the patient [11, 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 affect 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 GCF produced.

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 dose rate [26].

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 [11]. 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 [5, 26].

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.[2] 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 has been able to trigger the negative effects of the increase in GCF, which generates calculus formation induced by alkaline phosphatase enzyme from GCF and increases 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.

5. Conclusion

Panoramic radiography can cause an increase in the volume of GCF in Wistar rats with gingivitis and periodontitis. 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. Panoramic radiography can cause an increase in the volume of GCF in Wistar rats with gingivitis and periodontitis. 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.

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 affect 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 GCF produced. A panoramic radiograph that produces 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 [11].

Based on the study, the increasing number of GCF as a sign of the cell damage effect due to panoramic radiography can be minimized by further increasing the radioprotective effort of the patient. Increased radioprotective effort can be made by minimizing the possibility of technical errors when doing panoramic radiographs, thereby preventing the repetition of panoramic radiographic exposure and preventing the increase in dose absorbed by the patient [11,15].

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