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Research Report

Changes in the number of macrophage and lymphocyte cells in chronic periodontitis due to dental X-ray exposure

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ABSTRACT

Background: *Periodontitis is an inflammatory disease caused by specific microorganisms that attacks tooth-supporting tissues, P. gingivalis bacteria are mostly found in patients suffering from chronic periodontitis which is usually diagnosed by means of clinical and radiographic examination. The latter play important roles in the management of periodontitis, including: establishing diagnosis, determining treatment plans and evaluating the results of treatment. Unfortunately, the use of X-rays to perform such radiographic examination has negative effects since the body's various parts, especially the head, are not well protected from the effects of X-ray radiation.* ***Purpose:*** *This research aimed to analyze the effects of dental X-ray exposure on the number of macrophages and lymphocytes in experimental subjects suffering from periodontitis.* ***Methods:*** *36 rats that had been diagnosed with chronic periodontitis were divided into three groups, namely: a control group, treatment group I (exposed to a 0.16 mSv dose of radiation) and treatment group II (exposed to a 0.32 mSv dose of radiation). These subjects were subsequently sacrificed on the third and fifth days after treatment. Thereafter, histopathological examination was performed to identify any changes in the number of macrophages and lymphocytes.* ***Results:*** *The results of an HSD test confirmed that, on the third day, there were significant differences in the number of lymphocytes between the control group and treatment group I, as well as between the control group and treatment group II. On the fifth day, there were also significant differences in the number of lymphocytes between the control group and treatment group I, as well as between treatment group I and treatment group II. Similarly, there was a significant difference in the number of macrophage cells on the third day between the control group and treatment group I. On the fifth day, there were also significant differences in the number of macrophage cells between the control group and treatment group I, as well as between treatment group I and treatment group II.* ***Conclusion:*** *Dental x-ray exposure at a dose of 0.16 mSv can elevate the number of macrophages and lymphocytes on the third and fifth days. On the other hand, dental x-ray radiation at a dose of 0.32 mSv can reduce the number of macrophages on day 3 as well as the number of lymphocytes on the third and fifth days.*

Keywords: *dental x-ray radiation; macrophages; lymphocytes; chronic periodontitis*

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INTRODUCTION

Periodontitis is an inflammatory condition that affects tooth-supporting tissue caused by specific microorganisms and characterized by damage to periodontal tissue and alveolar bone surrounding the tooth.[1] Periodontitis is caused by gram-negative bacteria such as: *Porphyromonas gingivalis* (*P. gingivalis*), *Tannerella forsythia* (Tf) and *Actinobacillus actinomycetemcomitans* (Aa). However, the most common bacteria found in patients with chronic periodontitis are *P. gingivalis* at a percentage of 48%.[2] *P. gingivalis* contains lipopolysaccharide (LPS), toxic membrane vesicles and protease enzymes that play a role in inflammation.[3] Although bacteria and their bi-products

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play a role in periodontitis, the main causal factor of soft and hard tissue damage associated with periodontitis is that of the host immune response against the bacteria.[4]

The immune system components that contribute to periodontitis include macrophages and lymphocytes, the latter being immune cells characteristic of the host immune response to injury during chronic inflammation, whereas macrophages are immune and inflammatory cells that play an important role in host defense against periodontal pathogen infection.[5] Macrophages are crucial to the occurrence of non-specific immunity through the action of microbial phagocytosis and the production of cytokines which will then activate inflammatory mediators.[6] Several pieces of research have even shown that about 5-30% of inflammatory cells infiltrated in periodontitis tissue are macrophages, whereas the number of macrophages in gingival tissue suffering from periodontitis is higher than that in healthy gingival tissue.[7]

The diagnosis of periodontitis is carried out by means of clinical examination, including: establishing the extent of bleeding on probing, quantifying the depth of infection and radiographic analysis. The last mentioned is important in the management of periodontitis since it involves evaluating surrounding hard tissues and alveolar peak conditions, monitoring the extent of bone loss and increasing periodontal space. In addition, radiographic examination may also assist dentists in the areas of diagnosis, defining treatment plans and evaluating treatment outcomes. Unfortunately, the use of X-rays to produce radiography has a negative effect since certain parts of the body, especially the head, are not well protected from the effects of X-ray radiation.[8]

X-ray radiation is one form of radiation that can induce an ionization process in the media through which it passes. X-ray radiation used to produce intraoral radiographic images is administered at low doses ranging from 0.01 to 10 mSv.[9] Although included in low-dose radiation, the principle of radiation protection remains very important to the manufacture of radiographic images. This is because radiation at the lowest doses can still cause biological effects in the body due to x-ray ionization that can damage deoxyribonucleic acid (DNA).[9]

In the field of radiology, the radiation to which each patient is exposed is set at a dose limit value (NBD) required by the International Commission on Radiological Protection (ICRP). The limit value of radiation exposure doses received should not exceed 0.3 milisievert (mSv) per year. Meanwhile, the dose administered in a single periapical radiographic examination was 0.08 mSv.[10]

Nevertheless, the effects of dental X-ray radiation on patients with periodontitis have still not been fully explained. Therefore, the results of the research reported here are expected to reveal the effects of such exposure on the number of macrophages and lymphocytes in experimental subjects suffering from periodontitis.

MATERIALS AND METHODS

This research used 36 male Wistar rats aged 1.5-2 months and 150-200 grams in weight. These subjects were divided into three groups consisting of a control group, treatment group I and treatment group II, each consisting of 12 male Wistar rats. In the control group, the subjects were not exposed to X-ray radiation, while those in treatment group I were exposed to X-ray radiation at a dose of 0.16 mSv and those in treatment group II were exposed to X-ray radiation at a dose of 0.32 mSv. The research was conducted at the Laboratory of Biochemistry, Faculty of Medicine, Universitas Airlangga and at the Research Center of the Faculty of Dental Medicine, Universitas Airlangga.

Chronic periodontitis was subsequently induced in all subjects by the administering of 0.03 ml of *P. gingivalis* ATCC 33277 and 2 x 10^6 CFU/ml injected into the gingival sulcus of their lower right and left incisors once every three days for two weeks.[11] Clinical signs of chronic periodontitis, such as gingival hyperplasia, pocket formation

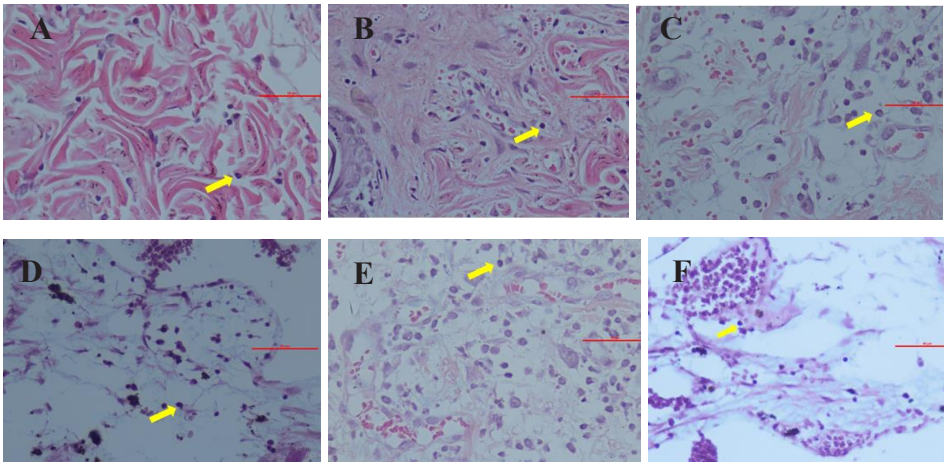

Figure 1. The results of HPA on the lymphocyte cells (yellow arrows) of the subjects suffering from chronic periodontitis using HE staining technique. (A) In the control group on day 3; (B) In the control group on day 5; (C) In treatment I group on day 3; (D) In treatment I group on day 5; (E) In treatment II group on day 3; (F) In treatment II group on day 5.

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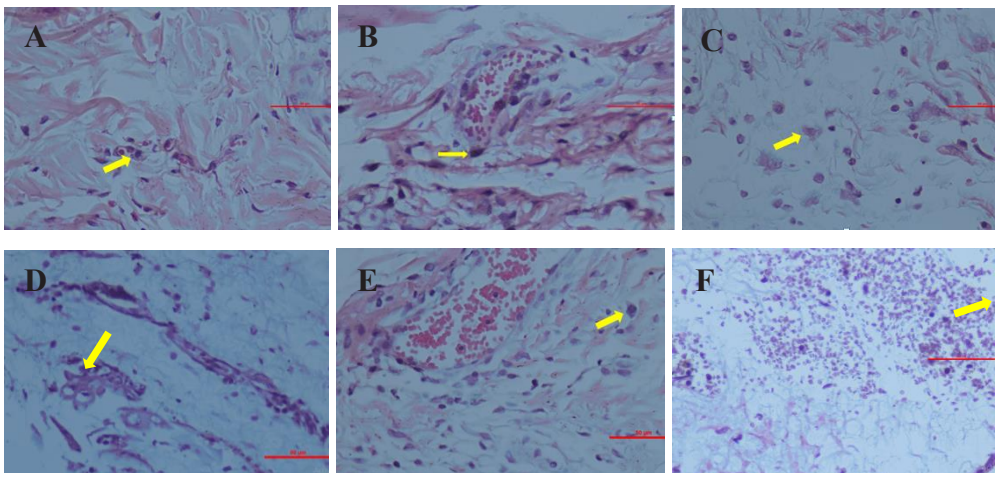

Figure 2. The results of HPA on the macrophage cells (yellow arrows) of the subjects suffering from chronic periodontitis using HE staining technique. (A) In the control group on day 3; (B) In the control group on day 5; (C) In treatment I group on day 3; (D) In treatment I group on day 5; (E) In the treatment II group on day 3; (F) In treatment II group on day 5.

Table 1. The mean value and standard deviation of macrophage and lymphocyte cell counts in the control group, treatment I group and treatment II group

		Control	Treatment I	Treatment II
Macrophages	Day-3	7.16 ± 1.16	10.5 ± 2.73	7.5 ± 2.42
	Day-5	8.33 ± 1.86	12.83 ± 3.43	8.5 ± 2.25
Lymphocytes	Day-3	2.16 ± 0.98	7.0 ± 1.78	6.16 ± 1.47
	Day-5	2.33 ± 0.98	9.0 ± 1.78	4.5 ± 1.04

Table 2. The results of a Kolmogorov Smirnov test and one-way ANOVA test on the number of macrophage cells on day 3

Groups	Significance of the normality test	Significance of the one-way ANOVA test
Control	0.4572	
Treatment I	0.9321	0.0378*
Treatment II	0.2729	

*p<0.05 there was a significant difference

and periodontal attachment loss were then observed. HPA examination was also carried out in order to confirm the diagnosis of periodontitis, after which treatment group I was exposed to a 0.16 mSv dose of X-ray radiation, while treatment group II was exposed to one of 0.32 mSv.

The subjects were sacrificed by means of ether inhalation on days 3 and 5 after treatment since on those specific days they would experience the initial and final phases of inflammation respectively. Their mandibular was then removed and fixed in 10% formalin solution. Thereafter, tissue processing was performed, prior to HE staining being conducted. The preparations were observed by using HE staining technique with a light microscope at a magnification of 400x from five (5) fields of view.

RESULTS

The results of the observation of the number of macrophages and lymphocytes can be seen in Table 1 and Figures 1 and 2.

The table above shows that the lowest number of macrophage cells was found in the control group sacrificed on day 3, but not exposed to X-ray radiation (7.16 ± 1.16). Meanwhile, the highest number of macrophage cells was in treatment group I exposed to X-ray radiation at a dose of 0.16 mSv and subsequently sacrificed on day 5 (12.83 ± 3.43). On the other hand, the lowest number of lymphocytes was found in the control group sacrificed on day 3, but not exposed to X-ray radiation (2.16 ± 0.98), whereas the highest number of lymphocytes was in treatment group I sacrificed on day 5 (9.0 ± 1.78).

Based on the results of the Kolmogorov Smirnov test illustrated in the table above, the number of macrophage and lymphocyte cells in all groups demonstrated normal distribution (p>0.05). The results of the one-way ANOVA test indicated the existence of a significant difference in the study groups. The HSD test results showed that there were significant differences between the research groups. For example, there was a significant difference in the number of macrophage cells on day 3 between the control group and treatment group I (p= 0.049). There were also significant

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Table 3. The results of a Kolmogorov Smirnov test and one-way ANOVA test on the number of macrophage cells on day 5

Groups	Significance of the normality test	Significance of the one-way ANOVA test
control	0.3577	
Treatment I	0.6085	0.0139*
Treatment II	0.3018	

*p<0.05 there was a significant difference

Table 4. The results of a Kolmogorov Smirnov test and one-way ANOVA test on the number of lymphocyte cells on day 3

Groups	Significance of the normality test	Significance of the one-way ANOVA test
control	0.0935	
Treatment I	0.5431	0.0001*
Treatment II	0.5245	

*p<0.05 there was a significant difference

Table 5. The results of a Kolmogorov Smirnov test and one-way ANOVA test on the number of lymphocyte cells on day 5

Groups	Significance of the normality test	Significance of the one-way ANOVA test
Control	0.0935	
Treatment I	0.5431	0.0001*
Treatment II	0.7624	

*p<0.05 there was a significant difference

differences in the number of macrophage cells on day 3 between the control group and treatment group II (p=0.963), as well as between treatment group I and treatment group II (p=0.08). Similarly, there were significant differences in the number of macrophage cells on day 5 between the control group and treatment group I (p=0.023) as well as between treatment group I and treatment group II (p=0.028). Meanwhile, there was no significant difference between the control group and treatment group II (p=0.993).

On the other hand, with regard to the number of lymphocyte cells, there were also significant differences on day 3 between the control group and treatment group I (p=0.0001) as well as between the control group and treatment group II (p=0.0006). However, there was no significant difference between treatment group I and treatment group II (p=0.592). There were significant differences in the number of lymphocyte cells on day 5 between the control group and treatment group I (p=0.001) as well as between treatment group I and treatment group II (p=0.001). However, no significant differences existed between the control group and treatment group II (p=0.108).

DISCUSSION

The results of the HSD test on day 3 showed that there was a significant difference in the number of macrophages between the control group and treatment group I. This indicates that when treatment group I was exposed to X-ray radiation at a dose of 0.16 mSv, the macrophage cells could still neutralize free radical damage. At the time of exposure, immune cells responded to the radiation exposure in the form of a wound so that activated macrophages proliferated more strongly in the affected area as the body's defense response. There was no significant difference between the control group and treatment group II or between treatment group I and treatment group II. However, the number of macrophage cells in treatment group II was higher than in the control group. This may be due to the higher dose of X-ray radiation (0.32 mSv), which causes the number of free radicals to increase and damage the chains of DNA, proteins, carbohydrates and macrophage cell lipids, leading to apoptosis of the macrophage cells. In addition, when the body responds to injury, it needs time to repair the damage resulting in inhibition of macrophage cell proliferation.[12]

The results of the HSD test on the number of macrophage and lymphocyte cells on day 5 revealed there to be significant differences between the control group and treatment group I as well as between treatment group I and treatment group II. The number of macrophages and lymphocytes was higher in treatment group I than in treatment group II and the control group. This could be caused by the radiation dose in treatment group I being lower than that in treatment group II so that the macrophage and lymphocyte cells in treatment group I could still improve the free radical damage and immediately proliferate in response to the injury. Meanwhile, in the second treatment group there was a decrease in the number of macrophages and lymphocyte cells. This may have occurred because the dose of X-ray radiation administered was higher so that macrophages and lymphocytes experienced a higher level

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of apoptosis than treatment group I. Radiation-induced cell apoptosis will intensify in tandem with the increase of the ionization radiation dose.[13]

Free radicals resulting from radiation can subsequently cause DNA damage such as the impeding of hydrogen bonds between chains, cross linking and breaking of DNA chains. Disruption of DNA will eventually lead to cell death or genetic mutations. X-rays can also interfere with cell mitochondrial function, resulting in the oxidation of carbohydrates, lipids and cell proteins. This, in turn, can induce disruption of the energy cycle in the cells. When free radicals cannot be neutralized by the body this can lead to inactivation of cell proliferation, retention of cell cycle check point, induction of cell apoptosis and inhibition of cell cycle.[12]

Chronic periodontitis is a disease in which the interaction between bacteria and the host immune response greatly affects the severity of the periodontal condition. Following the bacterial attack, an inflammatory reaction constitutes the body's defense response during which a number of immune cells such as macrophages and lymphocytes will congregate on the infected side. Macrophages represent one of the immune and inflammatory cells that play an important role in the host's defense against periodontal pathogen infection.[5] Meanwhile, lymphocytes are white blood cells that help the body's immune system fight infection. These cells produce antibodies against antigens in the inflammatory sites.[14] The presence of exposure to low to mid-dose X-ray radiation can then lead to apoptosis, whereas high-dose radiation will lead to the death of cells resulting in necrosis.[13]

Free radicals formed due to exposure to X-ray radiation can also cause intracellular stress which signals to the mitochondria causing them to undergo change. Transformation in the mitochondria begins in the open outer membrane, followed by swelling of the matrix and the loss of transmembrane potential which causes the mitochondria to lose their electron transport function. This leads to mitochondrial proteins such as Cytocrom-c being released. The detached cytocrom-c can then activate caspase 9 which may eventually lead to apoptosis.[13] Apoptosis that occurs in macrophages and lymphocytes can induce the immune system to work harder since, on the one hand, it must respond to the invasion of bacteria which, on the other hand, it must inhibit proliferation due to free radical effects caused by radiation. When immune cells undergo proliferative inhibition, the host's immune response to bacterial invasion will decrease which, in turn, will affect the body's defense mechanism to act against infectious diseases.[15]

Finally, it can be said that exposure to dental X-ray radiation potentially leads to changes in the number of macrophages and lymphocytes in rats suffering from periodontitis. Exposure to X-ray radiation at a dose of 0.16 mSv can increase the number of macrophages and lymphocytes on days 3 and 5, whereas exposure to X-ray radiation at a dose of 0.32 mSv can decrease the number of macrophages on day 3 and decrease the number of lymphocytes on days 3 and 5. Therefore, the principle of radiation protection is very important to consider in the manufacture of periapical radiographic images. Although the radiation used in producing such images is at relatively low doses, it can still cause cellular changes in the body since radiation at the lowest possible doses produces biological effects in the body.[9]

In conclusion, exposure to X-ray radiation at a dose of 0.16 mSv can increase the number of macrophages and lymphocytes on days 3 and 5, whereas exposure to X-ray radiation at a dose of 0.32 mSv can decrease the number of macrophages on day 3, while also reducing the number of lymphocytes on days 3 and 5.

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