

## RESEARCH ARTICLE

### Effect of $\beta$ -carotene patch application on gingival crevicular fluid volume after repeated periapical radiographic exposure

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#### ABSTRACT

Free radicals generated during ionization process of X-rays can damage biological tissues. Radiation exposure to gingival sulcus area will damage endothelial cells and increase permeability of blood vessels under sulcular and junctional epithelium. That inflammation will increase gingival crevicular fluid (GCF) volume. Repeated periapical radiographs often occurs due to the unfulfillment of quality assurance and leads to an increase amount of radiation dose received by the patient. Previous studies have shown that  $\beta$ -carotene mucoadhesive gingival patch can penetrate mucous membrane and provide protection against radiation by reducing the number of gingival epithelial cells micronuclei. The aims of this study was to observe  $\beta$ -carotene mucoadhesive gingival patch effect in GCF volume from patient exposed to repeated periapical radiographs. We recruited 10 participants from patients who receive repeated periapical radiographs in instalation of dentomaxillofacial radiology, Prof Soedomo dental and oral hospital Faculty of Dentistry UGM. The teeth of the subjects are divided into control and treatment group.  $\beta$ -carotene mucoadhesive gingival patch was applied to treatment group. GCF was collected using an absorbing paper strip before and after exposure, then measured by sliding caliper. Paired T-test showed significant differences ( $p<0.05$ ) between GCF volume before and after radiographic exposure in each group. Independent T-test showed significant differences ( $p<0.05$ ) of GCF volume between control and treatment group. Conclusion of this study is  $\beta$ -carotene mucoadhesive gingival patch significantly reduce GCF volume after repeated periapical radiographic exposure.

**Keywords:**  $\beta$ -carotene mucoadhesive gingival patch; GCF volume; repeated periapical radiography

#### INTRODUCTION

X-ray radiation can cause deterministic and stochastic effects on biological tissues. The deterministic severity effect is in line with the dose but has a threshold. If the dose is below the threshold, the response to the deterministic effect will not be performed. A stochastic effect explains the possibility of changes and has no threshold.<sup>1</sup> Damage due to X-rays is classified as direct and indirect damage. Direct damage occurs to specific targets in cells namely vital biological macromolecules such as DNA, RNA, proteins, or enzymes. Indirect damage to cells is due to free radicals produced by the ionization process of water or other cell molecules.<sup>2</sup>

Different cells in various organs of the same individual have different responses to radiation.<sup>3</sup> Vascular endothelial cells are more sensitive than

other types of mesenchymal cells. Endothelial cell permeability increases after radiation exposure.<sup>4</sup> The increased permeability of blood vessels under the sulcular and junctional epithelium is associated with the production of gingival crevicular fluid (GCF).<sup>5</sup>

Gingival crevicular fluid (GCF) is a physiological filtration product of a modified blood vessel, and originates from the blood so that its composition is almost the same as blood.<sup>6</sup> Ionization radiation can cause inflammation.<sup>7</sup> The inflammation has a direct impact on GCF production which causes an increased GCF flow so that the volume in the gingival sulcus increases.<sup>8</sup> Zuelkevin found an increase in gingival sulcus volume after exposure to panoramic radiographs.<sup>9</sup>

Periapical radiography is an intraoral technique which is commonly used in the field of

dentistry and serves to display images of individual tooth and surrounding apical tissue.<sup>2</sup> Ardakani and Dadsefat reported that the main cause of periapical radiographic errors and repetitions is technical errors by the operator when taking pictures and when processing.<sup>10</sup> In the field of dentistry, the radiation exposure dose used is relatively small.<sup>2</sup> Repetition of radiography increases the radiation dose received by the patient.<sup>11</sup> The higher the radiation dose, the effect of radiation will increase in terms of severity and frequency.<sup>12</sup> Radiation protection can minimize the effect of radiation exposure on patients and operators.<sup>13</sup>

Clinical studies showed that supplemental antioxidant such as  $\beta$ -carotene is able to provide radiation protective effects on body tissues.<sup>14</sup>  $\beta$ -carotene functions as an antioxidant to prevent free radicals that damage DNA cell.<sup>15</sup> Shantiningsih was able to show that  $\beta$ -carotene made in mucoadhesive gingival patch of  $\beta$ -carotene can penetrate mucous membranes.<sup>16</sup> Fajrian reported that the application of  $\beta$ -carotene mucoadhesive gingival patch can prevent an increase in the number of gingival epithelial micronucleus due to repeated periapical radiographic exposure.<sup>17</sup> Therefore, the study aimed to determine the efficacy of  $\beta$ -carotene mucoadhesive gingival patch to prevent an increase in GCF volume due to repeated periapical radiographic exposure.

## MATERIALS AND METHODS

The study was a quasi-experimental research. This study was declared to meet the research ethics requirements because it had obtained ethical approval in the form of issuing Ethical Clearance letters from the Ethics and Advocacy Unit of the Faculty of Dentistry, Universitas Gadjah Mada. Mucoadhesive gingival patch of  $\beta$ -carotene is a preparation obtained from previous studies. The composition consisted of HPMC,  $\beta$ -carotene, CMC-Na which was dissolved in distilled water and glycol propylene. Level of  $\beta$ -carotene in mucoadhesive gingival patch of  $\beta$ -carotene with a size of 3x10 mm or 30 mm<sup>2</sup> was 1.455-1.665  $\mu$ g.<sup>16</sup>

A total of 10 people as subject had meet the inclusion criterias such as willing to fill out and signed

informed consent, no history of periodontal disease and ulcers in the oral cavity, no history of systemic conditions/diseases that affect changes in GCF volume, no smoking, and no exposure of radiation at least 2 weeks before. The sample was divided into control and treatment groups taken from the same subject. The control group was a tooth that was not applied to the  $\beta$ -carotene mucoadhesive gingival patch and was on the right/left side of the tooth that was applied with a  $\beta$ -carotene mucoadhesive gingival patch with a distance of one tooth. The treatment group was a tooth that was subjected to periapical radiographic exposure and was applied to  $\beta$ -carotene mucoadhesive gingival patch before exposure.

Gingival crevicular fluid (GCF) sampling from subjects was carried out 30 minutes before exposure to the first periapical radiograph and 10 minutes after repeated exposure to periapical radiographs. Repetition of periapical radiographic exposure was performed if the image of the radiograph produced cannot show the information needed. The repetition was done once immediately on the same day with the first periapical radiograph exposure. GCF samples were taken using filter paper with intracrevicular technique; the tip of the filter paper was inserted into the gingival sulcus in the area of the tooth to be exposed to radiation until it touched the base of the sulcus. Two percent of ninhydrin solution was dropped on filter paper containing GCF until it turns blue or purple. The height and width of ninhydrin absorption and the thickness of filter paper were measured using a caliper. GCF volume was measured by multiplying the height of ninhydrin absorption with the absorption width and thickness of filter paper so that the volume obtained is expressed in mm<sup>3</sup> unit then the final result was converted to  $\mu$ L unit.<sup>5</sup>

Radiation exposure employed a periapical radiographic machine in the instalation of radiology dentomaxilofacial in Prof. Soedomo dental and oral hospital, Faculty of Dentistry Universitas Gadjah Mada, Siemens X-ray unit with specifications of 50 KV, 7 mA, and exposure time of 0.5 seconds. Data obtained from research were recorded in a table, and were analyzed using the Shapiro-Wilk

test to determine distribution normality. If the data is normally distributed, GCF volume data before and after radiation exposure from each group were tested using a paired t-test. Independent t-test was employed to compare the GCF volume differences between groups.

## RESULTS

The GCF volume in the first take (P1) and the second take (P2) of the control group are shown in Table 1 while GCF volume on the first take (P1) and the second take (P2) from the treatment group

are shown in Table 2. Table 1 shows an increase in the mean values of GCF volume after radiation exposure in the control group, however Table 2 shows a decrease in the mean values of GCF volume after radiation exposure in the treatment group. Based on Figure 1, it was seen that there was an increase in mean values of GCF volume after repeated exposure to periapical radiograph in the control group and a decrease in the mean of GCF volume after repeated exposure to periapical radiographs in the treatment group.

**Table 1.** Volume of gingival sulcus fluid of the control group

No.	P1 Control (μl)	P2 Control (μl)	Difference (μl)
1.	0.032	0.090	0.058
2.	0.182	0.249	0.067
3.	0.112	0.252	0.140
4.	0.072	0.158	0.086
5.	0.212	0.588	0.376
6.	0.010	0.365	0.355
7.	0.024	0.176	0.152
8.	0.190	0.142	-0.048
9.	0.160	0.468	0.308
10.	0.292	0.366	0.074
Mean ± SD	0.129 ± 0.094	0.285 ± 0.159	0.157 ± 0.142

Information:

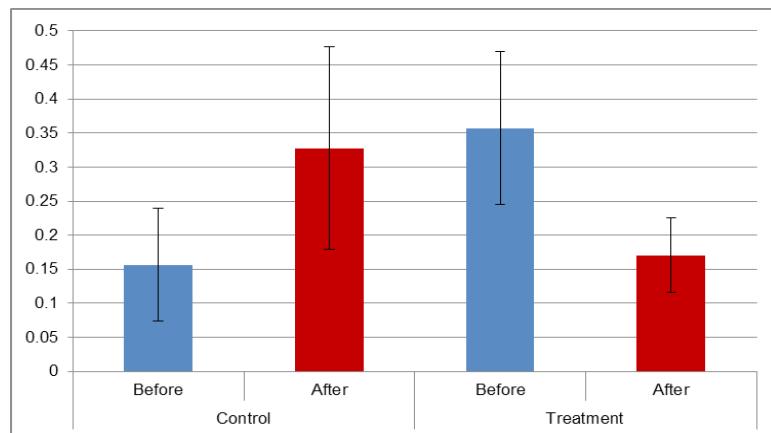
P1 Control: GCF Volume of control group before first exposure to periapical radiographs; P2 Control: GCF volume of the control group after repeated periapical radiographic exposure; Control Difference: P2 Control – P1 Control; Mean ± SD: Average ± standard deviation

**Table 2.** Volume of gingival sulcus fluid of the treatment group

No.	P1 Treatment (μl)	P2 Treatment (μl)	Difference (μl)
1.	0.461	0.179	-0.282
2.	0.389	0.222	-0.167
3.	0.270	0.177	-0.093
4.	0.278	0.144	-0.134
5.	0.458	0.210	-0.248
6.	0.350	0.158	-0.192
7.	0.236	0.061	-0.175
8.	0.207	0.130	-0.077
9.	0.363	0.161	-0.202
10.	0.560	0.262	-0.298
Mean ± SD	0.357 ± 0.113	0.170 ± 0.055	-0.187 ± 0.074

Information:

P1 Treatment: GCF Volume of treatment group before first exposure to periapical radiographs; P2 Treatment: GCF volume of the treatment group after repeated periapical radiographic exposure; Difference in Treatment: P2 Treatment – P1 Treatment; Mean ± SD: Average ± standard deviation



**Figure 1.** Graph of gingival fluid volume in the control and treatment groups (mean± SD)

**Table 3.** Paired t-test of GFC volume before and after repeated periapical radiographic exposure from each group

Data type	Paired t-test	
	t value	Significance (p)
The control GCF volume of the first take – The control GCF volume of the second take	-3.486	0.007
The treatment GCF volume of the first take – The treatment GCF volume of the second take	7.957	0.000

**Table 4.** Independent t-test of difference comparison between groups in GCF volume

Data type	Paired t-test	
	t value	Significance (p)
Difference in GCF volume of control group – Difference in GCF volume of the treatment group	6.771	0.000

Table 3 presents the GCF volume test results of the control and treatment group using the paired t-test. It shows that GCF volume before and after radiation exposure in each group had a significant difference ( $p<0.05$ ). Table 4 presents the result of the comparison of GCF volume difference between groups using the independent t-test and shows significant difference ( $p<0.05$ ) between the difference in GCF volume of the control group and the difference in GCF volume of the treatment group.

## DISCUSSION

The study of the  $\beta$ -carotene mucoadhesive gingival patch application on GCF volume due to repeated periapical radiographic exposure was carried out on

10 subjects. There was an increase in the mean of GCF volume after radiation exposure in the control group. The result is indicated by GCF volume data before and after radiation exposure in the control group. The results of the study are in accordance with the theory that biological disorders can occur indirectly against cells due to free radicals, one of which is produced by ionizing radiation from X-rays.<sup>13</sup>

The effective dose of radiography in the field of dentistry in one exposure is low, one of which is periapical radiography.<sup>18</sup> The risk of radiation that is more common in low doses is a stochastic effect, the effect that explains the possibility of a change and has no threshold.<sup>19</sup> Repetition of periapical radiography is frequently done and one of the

most common causes of the repetition is because the radiograph does not meet quality assurance requirements.<sup>20</sup> The radiation dose received by the patient will increase in line with radiographs image retrieval.<sup>11</sup> The higher radiation dose will increase the severity of the radiation effect.<sup>12</sup>

Previous study reported a significant increase in GCF volume after exposure to panoramic radiographs.<sup>9</sup> It is shown that repeated exposure to periapical radiographs could also cause a significant increase in GCF volume even though the increase was not as high as exposure to panoramic radiographs. The effective dose of periapical radiographs is 0.005 mSv while panoramic radiograph is 0.01 mSv.<sup>21</sup> However, the most effective dose of periapical radiographic exposure is 0.001 mSv while panoramic radiograph is 0.007 mSv.<sup>22</sup> The increase of GCF volume due to repeated periapical radiographic exposure is not as high as panoramic radiograph, it is probably because the dose of the periapical radiograph is lower than the panoramic radiograph.

The results of this study are also in line with the theory which states that radiation results in imbalance of cell regulation by several genes and factors involved in the inflammatory process.<sup>7</sup> Endothelial cells will experience increased vasodilation after exposure to radiation due to increased production of Inducible-Nitric Oxide Synthase (iNOS) which is responsible for producing Nitric Oxide (NO).<sup>23</sup> Vasodilation causes a loose bond between the sulcular and junctional epithelium, resulting in an increased flow of fluid from capillary arteries to connective tissue in response to the body. The mechanism causes an increase in GCF volume.<sup>8</sup>

The mean values of GCF volume after radiation exposure in the treatment group decreased. The result is indicated by GCF volume data before and after radiation exposure in the treatment group. These results are in accordance with the Okunieff et al. that antioxidants can reduce radiation cytotoxic effects on body tissues.<sup>24</sup>

The β-carotene protects radiation damage in human body tissue by X-rays through two mechanisms. The β-carotene antioxidant can

break the chain reaction by binding free radicals which cause the cessation of free radical activity so that it can prevent the harmful effects of free radicals in human body.<sup>25</sup> The antioxidant function of β-carotene is related to its molecular structure which allows binding of single oxygen, capturing the bonds chain of peroxide, and cleaning up free radicals.<sup>26</sup> β-carotene can inhibit the expression of the inducible-nitric oxide synthase (iNOS) and cikloxygenase (COX)-2 enzymes which are responsible for producing nitric oxide (NO) and prostaglandin E2 (PGE2) as mediators of inflammation. In addition, β-carotene can also inhibit the expression and production of inflammatory mediators such as TNF-α, IL-1β, IL-6, IL-12, and inflammatory genes in lipopolysaccharide-induced macrophages.<sup>27</sup>

It can be seen that there was a significant difference between GCF volume of the control group and the treatment group. These results indicate a significant effect of the application of β-carotene mucoadhesive gingival patch in preventing increased GCF volume after X-ray exposure on repeated periapical radiographic investigation. The study is in line with Shantiningsih that β-carotene in form of mucoadhesive gingival patch can penetrate mucous membranes and also functions as a protective material from radiation on panoramic radiographic techniques.<sup>16</sup> Fajrian stated that the application of β-carotene mucoadhesive gingival patch can also provide protection from radiation due to repeated exposure to periapical radiographs by preventing an increase in the number of gingival epithelial micronucleus.<sup>17</sup>

## CONCLUSION

The results showed that the β-carotene mucoadhesive gingival patch could prevent the radiation effect by reducing GCF volume significantly after repeated exposure to periapical radiographs.

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