# THE EFFECT OF CIGARETTE SMOKING ON VITAMIN C AND VITAMIN E LEVELS OF GINGIVAL CREVICULAR FLUID

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## INTRODUCTION

Recent studies indicate that immediate effect of smoking on gingival fluid flow produces a marked transient increase in gingival crevicular fluid (GCF), which may be a physiologic result of vasoconstriction and might reflect changes in gingival blood flow and in fluidity of cell membrane (1). Previous evidence demonstrated that gingival blood flow increased during smoking but the changes remained elevated for just 5 min after smoking (2,3).

Similar effects were obtained after smokeless tobacco (ST). ST users also showed elevated levels of $PgE_2$, IL-1$\alpha$ and IL-1$\beta$ in GCF compared to control subjects (4,5).

Evidence demonstrated that tobacco smokers have a more rapid rate of development of gingivitis than subjects who do not smoke tobacco (6). Experimental gingivitis in monkeys became worse when gingival blood flow was impaired by nicotinate ethyl ester treatment (7).

The development of gingivitis in smokers might be a consequence not only of nicotinic vasoconstriction but physical irritation of epithelial cell also. The purpose of this study was to assay the vitamin C and vitamin E levels in GCF of smokers and nonsmokers with clinically healthy gingiva.

## MATERIALS AND METHODS

Forty-one students, 18 males and 23 females (mean age of 23±1.6 years) were enrolled for this study. Subjects were in good general health and

did not require any drug treatment for at least one month before the recruitment. They were selected with no exclusion according to gender or ethnic background. Smokers were users of an average of 15 cigarettes per day at least for the past two years. All partecipants in the study gave informed consent to the experimental procedures. Individuals were divided in 25 tobacco smokers (15 males and 10 females) and 16 non tobacco smokers used as control subjects (3 males and 13 females). The periodontal status of both groups of subjects was assessed by a screening examination. All clinical assessments were carried out by the same investigator.

All volunteers were asked to brush their teeth after each meal at least three times a day. Oral hygiene was controlled every two days during the week before the experiment.

GCF samples were collected at midbuccal and midlingual sites of all teeth 3, 5, 6, and 7 of each of the following two regions: a) area of habitual cigarette placement, b) controlateral area of habitual cigarette placement. The same regions were examined in nonsmokers. GCF collection required appointments in three subsequent days. To avoid contamination with saliva, the teeth selected were isolated with cotton wool rolls and gently dried with air blasts. GCF was collected with paper strips, left in place for 3 min. The absorbed fluid volume was determined by a Periotron© 6000 which converted to microliter using a previously calculated standard curve calibrated using pooled human sera. The white part of the paper containing the fluid samples was cut off. All samples per tooth were collected and pooled in sealed plastic vials containing 100 $\mu$l buffered solution. The tubes were stored at -70°C before assay.

Frozen filter paper strips with GCF fluid samples were thawed and eluted by 50 ml of buffer for centrifugal elution (14.200 rpm at 4°C). The strips were eluted five times for 3 to 5 min each for a total collected volume of 250 $\mu$l.

Levels of vitamin C in the eluants were determined by HPLC (Serie 10 Perkin-Elmer, Italy) according to Rose and Nahrwold's method (8). Vitamin E levels were assayed by HPLC using the same procedure for the

analysis on plasma (9).

Measures obtained were recorded on a computer: correlation between the values obtained in smokers and nonsmokers and further statistical analyses were performed using statistical analysis system software (10).

RESULTS

The mean ascorbic acid concentration (219.3±64.9 μmol/l) in GCF of nonsmokers was significantly higher ($p<0.05$) than the corresponding GCF concentration of smokers (171.6±81.2 μmol/l). The two regions tested did not show any differences in vitamin C content of GCF in nonsmokers. The area of habitual cigarette placement of smokers showed a lower unsignificant ascorbic acid content compared to the controlateral area. The mean GCF tocopherol concentration of smokers did not reveal a significant low level in comparison of tocopherol values of nonsmokers, and differences between the two regions tested were not carried out. The vitamin contents of GCF in smokers and nonsmokers are summarized in table 1.

Tab. 1 - Ascorbic acid and tocopherol levels in GCF of 41 smoking and nonsmoking healthy students (M±SD)

| | Nonsmokers (16) | | Smokers (25) | | |
|---|---|---|---|---|---|
| | Area of cigarette placement | Controlateral area | Area of cigarette placement | Controlateral area | P value |
| Ascorbic acid (μmol/l) | 219.3±64.9 | 224.0±77.2 | 171.6±81.2 | 186.4±55.7 | 0.05 |
| Tocopherol (mg/l) | 4.5±0.8 | 4.9±0.3 | 3.7±0.4 | 3.9±0.2 | |

NS: not significant. P value: comparison between smokers and nonsmokers (paired t-test)

The decrease of ascorbic acid demonstrated that the smoke causes physical irritation on gingival vessels and this irritation is greater in area of habitual cigarette placement.

The decrease of tocopherol involves a reduction in the antioxidant properties and a concurrent epithelial gingival damage (11). Furthermore, the reduction of ascorbic acid causes a change of enzymatic mechanism continuously permitting tocopherol regeneration (12).

## DISCUSSION

Recent clinical trials showed that without tobacco abuse the effect of the antioxidative vitamins on redifferentiation of the oral mucosa was more intense.

Simultaneous serum measurements of vitamins such as C, E, A and $B_{12}$ demonstrated a significant decrease of oral leucoplakias compared to the control subjects (13,14).

The decrease of plasma antioxidant vitamins by tobacco smoking causes oxidative DNA modification implicated in certain damages of oral mucosal cells (15).

Experimental evidence on culture cells of chinese hamsters demonstrated the protective effect of alpha-tocopherol and ascorbic acid on chromosome aberration by tobacco agent compared to the controls (16).

Previous studies indicate that antioxidant vitamins may increase the resistance of the gingiva to local irritants and thus lead to a reduction in inflammation (17).

Subjects suffering with periodontal diseases showed low levels of ascorbic acid in GCF (18). This decrease can be ground for modifications of gingival mucosa in smokers through a long period of changes on gingival fluid flow and increased radical formation.

Clinical trial showed that vitamin E significantly augments endothelium-dependent relaxation in smokers (19). There are significant relationships between improvement in acetylcholine-induced vasodilatation and change in autoantibody title against oxidized LDL. Vitamin E reduces autoantibody levels against oxidized LDL increased in smokers. This study demonstrated

that tobacco smoking causes a reduction of alpha-tocopherol GCF levels and a significant decrease of GCF ascorbic acid value in students with clinically healthy gingiva.

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The purpose of this study was to assay the ascorbic acid and tocopherol levels in gingival crevicular fluid (GCF) of smokers and nonsmokers with clinically healthy gingiva. The comparison was determined between the area physically exposed to smoke and the controlateral area. All tested areas required to be free from periodontal diseases at a screening examination. 41 students (16 nonsmokers and 25 smokers) were enrolled in this study. GCF samples were collected in two regions: area of habitual cigarette placement and controlateral area. Areas sampled were at midbuccal and midlingual sites of all teeth 3, 5, 6, and 7. Ascorbic acid and tocopherol values of GCF were determined by HPLC. Smokers were found to have significant ($p<0.05$) lower levels of vitamin C in comparison to nonsmokers in all regions tested. Mean GCF tocopherol concentration of smokers did not reveal significant differences between the two regions examined. The vitamin A levels revealed an unsignificant low value in smokers in comparison to control subjects. Tobacco smoke can be the cause of a gingival damage by decrease of vitamin C and A operating through a vasoconstriction and a reduction of the antioxidant properties.

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