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# Salivary Levels Vitamin E and C in Different Histological Grading of Oral Cancer

## Níveis Salivares das Vitaminas C e E em Diferentes Gradações Histológicas do Câncer Oral

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### RESUMO

**Objetivo:** Avaliar os níveis salivares da vitamina C e vitamina E em pacientes com diferentes gradações histológicas do câncer oral.

**Método:** Níveis das vitaminas C e E antioxidantes foram estimadas em 15 pacientes de câncer oral com gradação II, 10 pacientes com gradação III e 12 pacientes com gradação IV. Estes tumores foram classificados de acordo com o critério da UICC. A saliva total não-estimulada dos indivíduos foi coletada sobre gelo e as amostras foram centrifugadas e congeladas a -20°C até a o momento da análise. Os níveis de vitamina C e E foram estimadas pela técnica HPLC. Todos os procedimentos foram realizados sob condições de luz protegida. Os resultados foram analisados pelo SPSS (7.0), e o teste t-student foi aplicado.

**Resultados:** Os níveis salivares das vitaminas C e E foram significativamente diminuídos em pacientes com grau histológico avançado de câncer oral ( $p < 0.001$ ). Houve uma significante correlação positiva entre a redução da vitamina C e vitamina E ( $r = 2.104$ ,  $p < 0.001$ ).

**Conclusão:** Os nutrientes antioxidantes podem ser utilizados em larga extensão em pacientes com câncer oral para contrair os radicais livres mediados por distúrbios celulares, o que resulta em redução dos níveis salivares antioxidantes.

### ABSTRACT

**Objective:** To assess the salivary levels of vitamin C and vitamin E in patients with different histological grading of oral cancer.

**Methods:** Antioxidant vitamin E and C levels were estimated in 15 patients of oral cancer grade II, 10 patients of grade III and 12 patients of grade IV. These tumors were classified according to UICC criteria. Unstimulated whole saliva from subjects was collected over ice and samples were centrifuged and frozen at -20°C until analysis. Vitamin C and vitamin E were estimated by HPLC technique. All procedures were performed under light protected conditions. Data was analyzed by SPSS (7.0), and student t-test were applied.

**Results:** The salivary levels of vitamin C and E were significantly decreased in patients with advancing histological grade of oral cancer ( $p < 0.001$ ). There was a significant positive correlation between reduction in vitamin C and vitamin E ( $r = 2.104$ ,  $p < 0.001$ ).

**Conclusion:** Antioxidant nutrients may be utilized to a greater extent in oral cancer patient to counteract free radical mediated cell disturbances, resulting in a reduction in salivary antioxidant levels.

### DESCRIPTORES

Câncer oral; Antioxidantes; Saliva.

### DESCRIPTORS

Oral cancer; Antioxidants; Saliva.

## INTRODUCTION

Lipid peroxidation may be involved in the process of oral cancer and essential nutrients that can scavenge free radicals, such as vitamin E and C, constitute a strong line of defense in retrading free radical induced cellular damage<sup>1</sup>. Free radical mediated lipid peroxidation may be involved in the cancer. It has been reported that increase in lipid peroxidation products in cancer and a decrease in antioxidant activity in cancer compared with normal<sup>2,3</sup>. Randomized controlled trials have shown that antioxidant (vitamin C and E) supplementation may be beneficial in prevention of cancer<sup>4,5</sup>.

Antioxidant nutrients counteract these free radical disturbances and, thereby protect cell membranes against free radical mediated lipid peroxidation<sup>1</sup>. Hence, the present study was planned to investigate the salivary levels of vitamin C and vitamin E in patients with different grading of oral cancer.

## METHODOLOGY

The 15 patients of oral cancer grade II, 10 patients of grade III and 12 patients of grade 4 (M:F, 25:25), age group 17-50 years attending out patient Department of Govt. Dental College associated with Pt. Bhagwat Dayal Sharma Postgraduate Institute of Medical Science, Rohtak (India). Tumors were classified according to UICC criteria<sup>6</sup>.

All diagnostic test evaluated for diagnosing oral cancer (Clinical grade II, III, IV). Unstimulated whole saliva from subjects were collected over ice and samples were centrifuged and frozen at  $-20^{\circ}\text{C}$  until analysis. Vitamin C and vitamin E were estimated by HPLC (under the grant aid from Dentsply, New Delhi).

HPLC separations were accomplished at room temperature (approx.  $37^{\circ}\text{C}$ ) with acecil liquid chromatography system (series, 1100, USA) consisting a sample injection valve with a 30  $\mu$  and sample loop, an ultraviolet (mv) spectrophotometric detector, a integrator and techsphere ODS-2 packed (4  $\mu$ m particle and 80.4 pore size) column (250x4.6 ID) with a methanol: acetonitril: chloroform (45: 41: 10 V/V) as mobile phase at 1 ml/min flow rate. All procedures were performed under light protected conditions. Data was analyzed by using SPSS (7.0), and student t-test was applied.

## RESULTS

The saliva levels of vitamin C were significantly decreased in patients with advancing grade of oral cancer ( $p<0.001$ ). Also, vitamin E levels were low in oral cancer in advancing grade ( $p<0.001$ , Table 1). There was a significant

positive correlation between fall in vitamin C and vitamin E ( $r=2.104$ ,  $p<0.001$ ).

**Table 1. Salivary vitamin C and E levels in various grade of oral cancer (Mean  $\pm$  SD).**

Parameters	Grade II	Grade III	Grade IV
Vitamin C (mg/ml)	1.23 $\pm$ 0.17	0.73 $\pm$ 0.19	0.62 $\pm$ 0.16
Vitamin E (mg/ml)	0.45 $\pm$ 0.18	0.29 $\pm$ 0.17	0.20 $\pm$ 0.17

$p<0.001$  at all levels.

## DISCUSSION

There exists a relationship between free radical activity and malignancy<sup>2,7</sup> and excess free radical disturbances are typically accompanied by increased utilization of antioxidants, resulting in decrease in their concentrations. In the present study, we observed a fall in both vitamin C as well as vitamin E levels in oral cancer with advancing grade (Table 1,  $p<0.001$ ). Water soluble antioxidant nutrients (reduced vitamin C) are initially consumed, followed by lipid soluble antioxidants (alpha tocopherol) in quenching the free radicals. Also, it has been reported that vitamin C regenerates vitamin E by non-enzymic mechanism<sup>8</sup>.

In addition, vitamin E and C have been demonstrated to inhibit superoxide production in the pig coronary artery suggesting that beneficial effects antioxidant vitamins are related, in part, to alterations in vessel redox status<sup>9</sup>. The decrease in antioxidant nutrient levels observed in this study (Table,  $p<0.001$ ) indirectly supports the concept that free radical mediated lipid peroxidation and related antioxidant consumption may be involved in pathophysiologic mechanisms of oral cancer.

Human defenses against oxidative stress and free radical damage primarily consist of antioxidant enzymes and nutrients. The antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) are synthesized in the body, and their concentrations cannot be easily influenced. In contrast, antioxidant nutrient levels can be simply manipulated by dietary or pharmacologic supplementation. The concept of increase utilization of vitamin C and E in patients raises the possibility of potential protective role for antioxidant nutrients in oral cancer.

## CONCLUSION

Antioxidant defences (vitamin E and C) are compromised and oxidative stress is increased in patients with oral cancer. A weak antioxidant defense system makes the mucosal cells more vulnerable to the cytotoxic effect of reactive oxygen species. This creates in intracellular

environment more favourable for DNA damage and disease progression. So, antioxidant supplement (vitamin C and E) may have role in oral cancer patients.

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