Assessment of Serum and Salivary Vitamin E Levels in Oral Potentially Malignant Disorders and Oral Cancer

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ORIGINAL ARTICLE

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ABSTRACT

An increase in lipid peroxidation and a decrease in antioxidant activity have been reported in patients with cancer in comparison with normal subjects. **Objective:** To estimate the status of serum and saliva by assessing the serum and salivary vitamin E levels in patients with oral potentially malignant disorders and oral cancer. **Methods:** A total of 90 participants were enrolled in this study. 30 subjects with oral potentially malignant disorders, 30 subjects with oral cancer, and 30 healthy subjects (controls). Serum and saliva samples were collected and vitamin E levels were assessed. The data were analyzed using ANOVA for between-group comparison. Post hoc Tukey analysis was used for comparing the two study groups with the control group. Pearson correlation coefficient was used to determine concordance between the groups. **Results:** Mean vitamin E levels in serum and saliva were significantly decreased in oral potentially malignant disorders and oral cancer compared with controls. **Conclusion:** As significant reduction in vitamin E levels was observed in saliva, it was evident that salivary vitamin E levels potentially be used as a reliable, non invasive biomarker for diagnosing and managing oral potentially malignant disorders and oral cancer.

Keywords: antioxidants; lipid peroxidation; saliva; serum; vitamin E

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INTRODUCTION

Oral cancer or oral cavity cancer, a subtype of head and neck cancer, refers to any cancerous tissue growth located in the oral cavity. The oral cavity encompasses the lips, buccal mucosa, retromolar trigone, hard palate, alveolar ridges, floor of the mouth, and anterior two-thirds of the tongue.[1]As the oral cavity is the portal to the respiratory and alimentary tracts, it has an increased exposure to numerous carcinogens, primarily alcohol, tobacco, betel nut, and human papillomavirus.[2] The incidence and mortality of oral cancer varies on the basis of the geographic location. An increased proportion of younger patients were diagnosed with oral cancer, especially tongue cancer, in the past decade.[3] The worldwide scenario reveals that males are primarily affected by oral squamous cell carcinoma at a ratio of 1.5:1. But the sex difference normalizes in areas where females consume tobacco and betel nut.[4]

Lipid peroxidation maybe associated with the processes involved in oral cancer. Free radical scavengers, such as vitamin C and vitamin E, constitute a powerful line of defense, which delays free radical-induced cel-
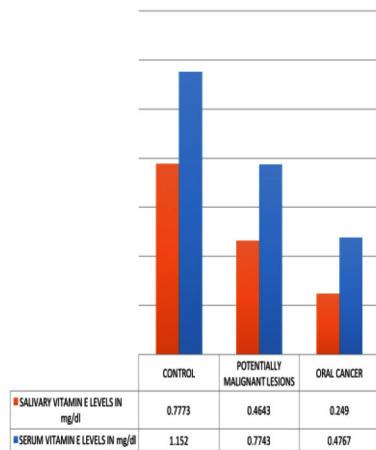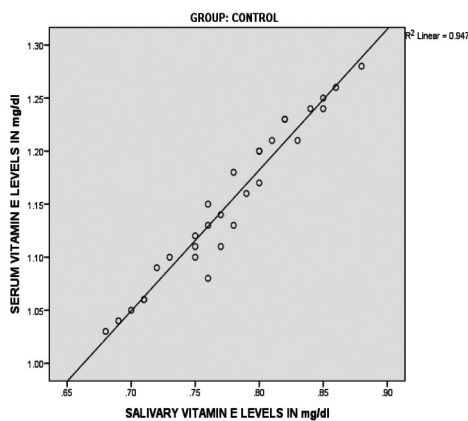
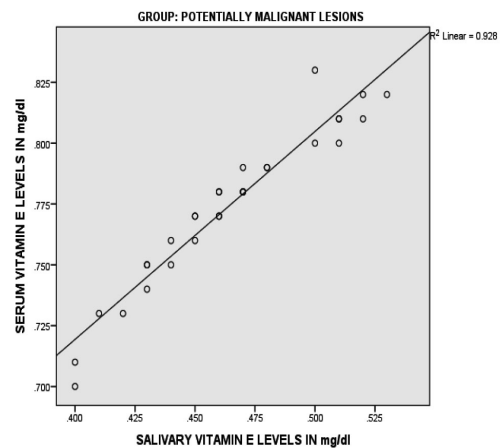**Figure 1.** Mean serum and salivary vitamin E level

Figure 3. Correlation of vitamin E levels in serum and saliva of subjects with potentially malignant disorders (R^2 Linear = 0.928)

Figure 2. Correlation of vitamin E levels in serum and saliva of controls subjects (R^2 linear = 0.947)
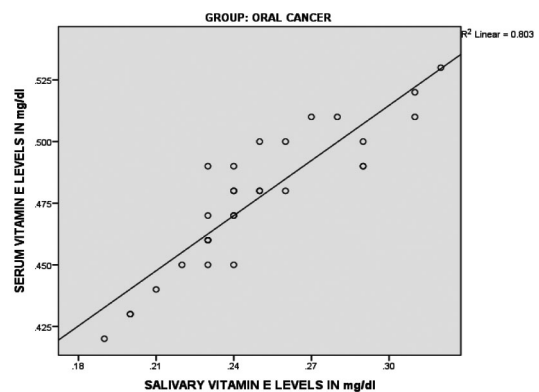

Figure 4. Correlation of vitamin E levels in serum and saliva of subjects with oral cancer (R^2 Linear = 0.803)

lular damage. Antioxidant defenses become depleted, which result in increased oxidative stress in patients with oral cancer. When the antioxidant defense system is weakened, the mucosal cells are more susceptible to the cytotoxic effect of reactive oxygen species. Therefore, we attempted to evaluate the status of serum and saliva levels of vitamin E. We believe this research will also help in establishing salivary vitamin E as a non invasive biomarker.[5]

METHODS

This was an observational study that was conducted in the Department of Oral Medicine and Maxillofacial Radiology of a Dental College in South India. This study was approved by the University's ethics committee. Written informed consent was obtained from the participants prior to data collection. The study sample consisted of 90 participants aged 30-60 years, including 27 females and 63 males who were divided into

three groups of 30 patients each. The control group (group I) included 30 healthy subjects without any oral lesions. One study group (group II) comprised 30 histopathologically confirmed patients with oral potentially malignant disorders such as oral leukoplakia, oral submucous fibrosis, and erosive lichen planus, and the other study group (group III) included 30 patients who were histopathologically diagnosed with oral squamous cell carcinoma.

Sample Collection
Ethical clearance was obtained from the concerned authorities, and written informed consent was obtained from all participants. Detailed case history was recorded, and the oral cavity was thoroughly examined in all cases.

Saliva Collection
Saliva samples were collected from patients 2 hours post their last food ingestion. Unstimulated saliva was collected using the spit technique[6] in which the patient

Table 1. Post hoc Tukey analysis

Dependent variable	I Group	J Group	Mean difference (I-J)	Std. Error	p
	Control	Potentially malignant lesions	.37767	.01255	<0.001
		Oral cancer	.67533	.01255	<0.001
Serum Vitamin E Levels in mg/dL	Potentially malignant lesions	Oral Cancer	.29767	.01255	<0.001
	Control	Potentially malignant lesions	.31300	.01083	<0.001
		Oral cancer	.52833	.01083	<0.001
	Potentially malignant lesions	Oral cancer	.21533	.01083	<0.001

was asked to sit on the dental chair with the head tilted forward with instructions not to speak or swallow any saliva. The patient was then asked to spit in a sterile graduated container every minute for 10mins.[7]

Blood Collection
In total, 5ml of blood was collected from the antecubital vein of each patient. The blood was collected using glass vials containing 3% citric acid, and serum was extracted and stored in glass vials at +4°C.

Method of Estimating Vitamin E
Vitamin E quantitatively converts ferric ions to ferrous ions, which forms an orange-colored complex when combined with bathophenanthroline. After adding phosphoric acid to stabilize the complex, the color is read at 536 nm.[8]

Overall, 1mL of ethanol solution was pipetted into a 4mL glass test tube for analysis. At the same time, standard preparations of 1, 2, 5, and 10μg of α-tocopherol were made up to 1mL in 100% ethanol and 0.2mL of 0.2% bathophenanthroline in ethanol was added. The contents of each tube were thoroughly mixed. The assay proceeded rapidly and care was taken to not expose the solutions to direct light. Next, 0.2mL of a 0.001M $FeCl_3$ solution in ethanol was added, followed by mixing with a Vortex mixer. After 1 min, 0.2mL of 0.001M H_3PO_4 solution in ethanol was added, and the contents of the tubes were again thoroughly mixed.[8]

Method of Analysis
The data obtained from the present study were analyzed using SPSS statistics software version 18. Data were analyzed using ANOVA for comparing between the groups. Post hoc Tukey analysis was used for comparing the two study groups with the control group. Pearson correlation coefficient test was used for determining concordance between the groups.

RESULTS

Mean serum and salivary vitamin E values are depicted in Figure 1. Comparative analysis of the study groups with the control group using posthoc Tukey analysis is shown in Table 1, and correlation between all of the groups is depicted in Figures 2, 3, and 4.

DISCUSSION

Vitamin E is composed of eight different stereoisomers, one of which is α- tocopherol, which is the most potent lipophilic antioxidant in vitro; another major factor is the interaction among antioxidants. Alpha-tocopherol is an effective peroxyl free radical scavenger, while ascorbic acid forms the prime defense line against aqueous peroxyl radicals. For synergistically combining these two antioxidants, either the induction phase or the lag phase should be longer than the sum of the induction periods produced by the individual antioxidant, or the rate of oxidation inhibited by the two antioxidants should be similar or smaller than that inhibited by either of the antioxidants. The combination of ascorbate and α-tocopherol efficiently prevents oxidation.[8]

Epidemiological studies indicate an inverse relationship between the risk of certain cancers and the consumption of fruits and vegetables containing essential antioxidant micronutrients, other essential micronutrients, phytochemicals, and fiber. In addition, an inverse risk has been shown for cancers of the oral cavity.[9] Vitamin E conserves the integrity of the membrane, inhibits cancer cell growth and differentiation and also obstructs mutagenicity and nitrosamine formation. The synergism among vitamin E, ascorbate, and selenium inhibits DNA and RNA protein synthesis in the cells.[10]

In the present study, mean serum vitamin E levels inpatients with oral potentially malignant disorders were significantly lower than that in controls, which is in agreement with a study by Gupta et al on oral submucous fibrosis. This could be attributed to the potent lipophilic action of vitamin E, which gets consumed in conditions of oxidative stress, thus lowering its levels.[11]

Furthermore, we observed a significantly reduced mean serum vitamin E levels in patients with oral cancer compared with that in controls, which is in accordance with the findings of a study by Raghuwanshi et al.[12] Thus, lower vitamin E levels may be caused by increased antioxidant utilization s by the tumor tissues

or to counteract free radical-mediated cell disturbances.[12,13]

Few salivary studies have been documented in the literature. In the present study, the mean salivary vitamin E values in patients with oral potential malignant disorders were significantly reduced compared with that in healthy controls, which is in agreement with studies by Chandra et al. and Rai et al., which state that antioxidant defenses are compromised and result in increased oxidative stress; therefore, antioxidant vitamins maybe utilized for counteracting free radical-mediated cell disturbances.[14,15]

CONCLUSION

Our study findings indicate reduced vitamin E levels in serum and saliva and thus illustrate their protective action against lipid peroxidation mediated by free radicals. However, further research on salivary vitamin E levels in oral potentially malignant disorders and oral cancer is still required.

CONFLICT OF INTEREST

The authors have no financial interests related to the material in the manuscript.

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