Original Article

Estimation of salivary nitric oxide and uric acid levels in oral squamous cell carcinoma and healthy controls

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ABSTRACT

Background: Oral squamous cell carcinoma (OSCC) being the most common head and neck cancer, involves the interplay of several free radicals and antioxidant molecules. The potential role of salivary nitric oxide (NO) and uric acid in cancer development needs to be explored as there are a few studies highlighting their association with each other and with oral cancer. **Aims:** The present study was designed to measure the NO and uric acid levels in the saliva of patients with OSCC as compared with healthy controls and to highlight any possible correlations between them. **Materials and Methods:** The present study involved 50 subjects, 25 with OSCC (study) and 25 healthy individuals (controls). Saliva samples collected from patients were subjected to NO and uric acid analysis by griess method and uricase method, respectively. **Statistical Analysis:** The results were analyzed using Student's *t*-test and Pearson's Chi-square test. **Results:** A significant increase in the salivary levels of NO was seen in study subjects as compared to healthy controls. On the contrary, a significant decrease in salivary uric acid level was observed in the study group as compared to healthy controls. In addition, there exists an inverse correlation between NO and uric acid levels in study and control groups. **Conclusion:** Salivary levels of NO and uric acid may act as key bimolecular markers in the detection of oral cancer, which could be further confirmed by larger sample size and future studies.

Key words: Nitric oxide, oral squamous cell carcinoma, saliva, uric acid

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is one of the most common cancer affecting humans with high morbidity and mortality rate. Free radicals in saliva that induce oxidative and nitritive stress are principal inducers of OSCC.[1] These free radicals are normally neutralized by efficient systems in the body that include antioxidant molecules.[2] Antioxidants are present in all body fluids and tissues, protecting against endogenously formed free radicals.[3] In healthy individuals, a delicate balance exists between free radicals and antioxidants, however, in oral cancer, this balance seems to be disturbed.[3] Nitric oxide (NO) is one such potent free radical produced in the presence of NO synthase (NOS) enzyme in the plasma, which is later concentrated into the saliva, and which mediates several physiological and pathological processes.[4] The overproduction of NO leads to cellular mutational events resulting in carcinogenesis (tumor formation).[4] Chemicals present in the oral microenvironment are seen to be dominantly controlling the mutational events in a cell leading to carcinogenesis as compared with genetic changes.[4] On the other hand, uric acid is a free

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radical-scavenging antioxidant that scavenges radicals to inhibit chain initiation and break the chain propagation. It forms the most important antioxidant in saliva contributing to about 85% of total antioxidant capacity.[5,6] Urate being the most predominant salivary antioxidant could possibly be a better indicator of uric acid production in the body as compared to other body fluids.[2] Reactive nitrogen species (RNS) associated with inflammation in the oral cavity can lead to accumulation of nutritive stress due to lack of antioxidant defense mechanism which ultimately results in DNA damage and carcinogenesis.[6]

The purpose of the present study was to evaluate the levels of NO, which possibly contributes to tumor formation and metastasis and the inhibitory action of uric acid, which helps prevent these changes, in the saliva of patients with OSCC and controls.

MATERIALS AND METHODS

Three milliliter of whole saliva was collected in the daytime between 9 am and 11 am from a group of 25 otherwise healthy and consenting OSCC patients (study group), and 25 consenting healthy individuals without any risk habits (control group). These individuals were selected among the patients visiting the Department of Oral Medicine and Radiology in the institute. Data regarding the personal history, medical, dental, habit history were recorded in appropriate case history format. Inclusion criteria: Otherwise systemically healthy individuals without the presence of gout, renal, vascular, and cardiovascular diseases. Exclusion criteria: Patients who have taken antibiotics, anti-inflammatory agents, immunosuppressant drugs, or on treatment for oral cancer in previous 6 months. Saliva samples were subjected to the estimation of NO and uric acid by griess method and uricase method, respectively. Subjects were asked to rinse their mouth with 3 ml of normal saline for 3 min. The individual was then asked to spit in a sterile beaker, and the collected sample was sent for biochemical analysis.

Estimation of nitric oxide by griess colorimetric method

Reagents

- Sulfanilamide solution
- N-(1-naphthyl)-ethylenediamine dihydrochloride solution
- Colorimeter.

Principle

The estimation of NO was done by standard kit (Agappe Diagnostics, India Ltd). NO concentration is based on the enzymatic conversion of nitrate to nitrite by colorimetric detection of nitrite as an azo by-product of the Griess reaction. The Griess reaction is based on the two-step diazotization reaction in which acetified NO produces a nitrosating agent, which reacts with sulfanilic acid to produce diazonium ion. This ion is then coupled to N-ethylenediamine to form the chromophoric azo derivatives, which absorb light at 540–570 nm. The measurement of absorbance at 550 nm is read after 10 min. The concentration is calculated from a calibration plot prepared from a series of standard nitrite.

Estimation of uric acid by uricase colorimetric method

Reagents

- Phosphate buffer
- Ferrocyanide
- Uricase
- Peroxidase
- 4-aminoantipyrine (4-AAP)
- Colorimeter.

Principle

The estimation of uric acid was done by standard kit (Agappe Diagnostics, India Ltd), which contains buffer reagent and color reagent. The hydrogen peroxide, liberated by the action of Uricase, reacts with peroxidase and 4-AAP to form a colored product. The absorbance of the colored product is measured at 548 nm using colorimeter.

Student's *t*-test was used to compare mean levels of NO and uric acid in study and control groups. Pearson correlation test was used to correlate the levels of both NO and uric acid in study and control group. $P < 0.05$ was considered as statistically significant.

RESULTS

The mean NO level in subjects with squamous cell carcinoma (study group) was 88.13 μM whereas, in the control group was 27.15 μM. On the other hand, the mean uric acid level in subjects with squamous cell carcinoma was 2.03 mg/dl and in controls was 5.38 mg/dl [Table 1].

The study group had a significantly higher level of NO as compared to control group with mean difference of 60.97 μM (95% confidence interval [CI] 51.26–70.69, $P < 0.001$), whereas the uric acid levels in study group were significantly lower as compared to control group with mean difference of − 3.76 (95% CI − 3.76–[−2.93], $P < 0.001$) [Table 2].

Overall a significantly strong inverse correlation was observed between NO and uric acid levels in the study participants ($r = -0.819$, $P < 0.001$). When the correlation between NO and uric acid levels was assessed in study and control groups separately they were not statistically significant ($P > 0.05$) [Table 3].

Table 1: Mean uric acid and NO levels in study and control groups

	Group	*n*	Mean	SD
Uric acid level (mg/dl)	Cases	25	2.03	0.80
	Controls	25	5.38	0.64
NO level (μM)	Cases	25	88.13	23.37
	Controls	25	27.15	3.06

SD: Standard deviation, NO: Nitric oxide

Table 2: Comparison of uric acid and NO levels in study and control groups

	Mean difference	95% CI of the difference		*t*	df	*P*
		Lower	Upper			
Uric acid level (mg/dl)	−3.35	−3.76	−2.93	−16.201	48	<0.001*
NO level (μM)	60.97	51.26	70.69	12.93	24.82	<0.001*

Student *t*-test, **P*<0.001 statistically significant. CI: Confidence interval, NO: Nitric oxide

Table 3: Correlation in the levels of both uric acid and NO in study and control group

Uric acid level (mg/dl)	NO level (μM)
Pearson correlation	−0.819
Significant (two-tailed)	<0.001*
n	50

**P*<0.05 statistically significant, *P*>0.05 nonsignificant. NO: Nitric Oxide

DISCUSSION

Nitric oxide is a free radical with an unpaired electron that is produced in the body by the isoenzyme NOS using L-arginine as a substrate. NO may mediate DNA damage through the formation of carcinogenic nitrosamines, generation of RNS and inhibition of DNA damage repair mechanism and can thus be considered as a tumor initiating agent.[7] Salivary levels of NO are caused due to absorption of dietary nitrates from the upper gastrointestinal tract and actively concentrated from the plasma into the saliva through an active transport system in the salivary glands.[1] Ohashi *et al*. noted the effects of NO on cultured cells using NO donating agents and found that it caused severe damage to fibroblasts, keratinocytes, and oral epithelial cells *in vitro*.[8] This could possibly be an explanation for the association of high levels of salivary NO with ulceroproliferative lesions in OSCC. Various other studies also have found high levels of salivary NO in OSCC cases as compared to healthy controls indicating its potential role in promoting carcinogenesis through genetic change.[1,3,4,8-12] Our findings have been consistent with previous studies and have shown similar results with regards to mean levels of NO. However, *in vitro* experiments by Shang *et al*. have found that NO donor drugs induced apoptosis of oral cancer cells at higher concentrations, and eventually caused direct cytotoxicity and cell death.[4,13] This could be another possible explanation for the elevated levels of salivary NO seen in oral cancer which could be the body's natural protective response against tumor cells.

Uric acid is a major antioxidant derived from the oxidation of xanthine and hypoxanthine by xanthine oxide reductase which neutralizes free radicals.[2] Approximately, about one-third of uric acid in the body is excreted through urine and intestinal secretions to undergo uricolysis. Diurnal variations in the elimination of uric acid cause variable patterns in salivary uric acid excretion with high levels seen during sleep. Hence, salivary urate estimation is noninvasive and acceptable to patients than blood or urine.[14] A plethora of studies on salivary levels of uric acid has shown that it is the most important antioxidant which decreases in the saliva in oral cancer as compared to healthy controls.[6,15-20] Diet derived availability of various antioxidants in saliva either directly or indirectly correlate with protection against oxidative stress.[17] Our study also reflects the findings which have been obtained in other studies showing lower mean levels of uric acid as compared to healthy controls.

A few studies have established a direct link between free radicals and antioxidant levels showing an inverse correlation[1] and our findings are in agreement with the findings mentioned in these studies. The present study emphasizes the potential role of salivary NO and uric acid levels in the saliva of patients with oral cancer thus encouraging their easy and quick assessment in the laboratory, but future studies with larger sample size may be beneficial to support the above findings.

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Conflict of interest

There are no conflict of interest.

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