



ESTIMATION OF URIC ACID IN THE SALIVA OF PATIENTS WITH ORAL CANCER AND ODONTOGENIC CYSTS: A BIOCHEMICAL STUDY

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ABSTRACT

Background: Oxidative Stress, which is related with chronic inflammation can predispose tissue to cancer development. There are some reports in the literature concerning primary tumors such as squamous cell carcinoma arising from odontogenic cysts. The reason for this transformation is still unknown. Saliva is rich in antioxidant compounds and is considered to be the first line of defense against oxidative stress, the main cause for many systemic and oral health diseases. The primary antioxidants include uric acid, albumin, ascorbic acid, glutathione and antioxidant enzymes with uric acid, being the most important antioxidant molecule in saliva contributing approximately to 70% of the total antioxidant capacity.

Aim and objective: To verify the utility of uric acid concentration in saliva in differentiation of healthy subjects, people with oral squamous cell carcinoma and odontogenic cysts, by using semiautomatic analyser.

Material and method: Unstimulated whole saliva was collected in a sterile container after overnight fasting from ten each of age matched Healthy subjects (Group 1), patients diagnosed with Oral squamous cell carcinoma (Group 2) and Odontogenic Cyst (Group 3). The concentration of uric acid in saliva was determined using a 'ROASCH URIC ACID' assay kit.

Results: The salivary uric acid concentration significantly lowered from 6.4mg/dl in healthy control to 3.3mg/dl in odontogenic cyst to 1.8 mg/dl in oral squamous cell carcinoma. The present study adds an evidence that lowered level of salivary uric acid concentration can be considered as an important mechanism by which toxic effects of free radicals can initiate malignant transformation of cyst.

KEYWORDS : antioxidants, odontogenic cyst, oral squamous cell carcinoma, oxidative stress, oxidants, saliva, uric acid.

INTRODUCTION

Oral Cavity Cancer is one of the most common malignancies of the head and neck with major risk factors being tobacco and alcohol, nutritional factors, chronic inflammation caused by chemical irritation, bacterial or viral infections. It may also arise from odontogenic cysts- inflammatory periapical, residual, dentigerous cysts and keratocyst odontogenic tumor with most of the carcinomas arising in odontogenic cysts being histopathologically well differentiated.^[1]

Some researchers have indicated lower antioxidant status, long standing chronic inflammation, continuous intracystic pressure, increased keratinisation of epithelial lining in the cyst might have been one of the causes of malignant transformation.^[2]

Inflammation is associated with enhanced generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The imbalance between the production of ROS and antioxidant defense system results in oxidative stress. A lack of detoxification or damage repair factors may cause oxidative DNA damage and carcinogenesis.^[3]

Saliva represents first line of defense against oxidative stress and contains antioxidants like uric acid, ascorbic acid, albumin and various enzymes like superoxide dismutase, glutathione peroxidase, catalase.^[4] Lower total antioxidant capacity was exhibited by elderly,^[5] heavy smokers,^[6] premalignant lesions^[7] and periodontal disease.^[8]

The role of biochemical markers, on the other hand, comes out to be a convincing enough evidence of the changes taking place in the body at a time when tissue and cell level changes are not obvious for frank malignant degenerations.

Estimating uric acid considered as dominant antioxidant present in the saliva could be helpful as an important diagnostic adjunct in the early diagnosis of oral cancers as against the other invasive and not so cost effective diagnostic adjuncts. Also, can assist in possible early identification and even more significantly, in determining the pre-disposition of the various odontogenic cysts as they transform into frank oral cancers.^[9]

Various experimental studies have been conducted to prove that uric acid concentration decreases in oral cancer patients and it is confirmed that free radicals increase and antioxidants decrease in oral squamous cell carcinoma.^[1,10,11,12,13] Literature search, revealed only one study[1]

estimating uric acid in Oral Cavity Cancer and Odontogenic Cyst using spectrophotometer hence, the present study was conducted.

MATERIALS AND METHODS

The present study included 30 individuals divided into 3 groups. Group 1 comprised of 10 age matched healthy individuals with no habits and systemic diseases, Group 2 comprised of 10 histopathologically confirmed cases of patients with oral squamous cell carcinoma (well differentiated (4) OSCC, moderately differentiated (4) OSCC, poorly differentiated (2) OSCC), Group 3 comprised of 10 histopathologically confirmed cases of odontogenic cysts ((5) periapical cyst, (5) dentigerous cyst) reported to the OPD of Pacific dental college and hospital were included. The mean and standard deviation value of age in Group 1 was 35.9 and 12.22, in group 2 was 45.2 and 11.72 and in group 3 was 35.9 and 12.22 respectively. In group 1 among healthy individuals 4 were females and 6 were males, in group 2 among OSCC patients 7 were males and 3 were females, in group 3 among odontogenic cysts patients 5 were males and 5 were females. Ethical clearance from the ethical committee of the institute and informed consent from the patients were taken.

EXCLUSION CRITERIA: Patients with periodontal disease, recurrent aphthous ulcers, premalignant lesion, antioxidant medications. Also patients who were chronic alcoholics and smokers were excluded from the study to rule out the probability of variation in the salivary uric acid level.

INCLUSION CRITERIA: Patients with newly diagnosed cases, no therapeutic modalities and without systemic diseases were included
SALIVA SAMPLE: Patients were instructed to do overnight fasting. Next day between 8 a.m. to 10 a.m. patients were asked to rinse their mouth with distilled water to wash out exfoliated cells. After washing for 1 minute, approximately 5 ml freshly secreted, unstimulated whole saliva was collected in a sterile container. The saliva was centrifuged (10,000 rpm, 10 mins) and the supernatant fluid was stored in the ice until it was used.

URIC ACID QUANTIFICATION: Uric acid concentration in the saliva was determined with the help of 'URIC ACID ROCHE' ASSAY which is a slight modification of the calorimetric method using COBAS C 111 semiautomatic-analyser machine.

STATISTICAL ANALYSIS: The data was analysed by one-way

analysis of variance (ANOVA) and post hoc test (Bonferroni test) using SPSS Statistical Package for significance of differences between each group. The p value less than 0.05 was considered to be statistically significant.

RESULTS

The mean values of salivary uric acid concentration lowered from 6.4 mg/dl in healthy subjects to be low as 3.3mg/dl in odontogenic cysts and 1.8 mg/dl in OSCC patients with statistically significant results (p value 0.02) as shown in (Table 1).

PARAMETER	GROUPS	N	MEAN±SD	p Value
SALIVARY URIC	Healthy Subjects	10	6.4± 0.82	
ACID	Odontogenic cysts	10	3.3± 1.14	0.02
(mg/dl)	Oral squamous cell carcinoma	10	1.8±0.80	

Also The mean value of salivary uric acid concentration lowered from (2.9±0.29) in well differentiate OSCC to be low as (1.3±0.26) in moderately differentiated OSCC and (1.2± 0.25) in poorly differentiated with statistically significant results (p value 0.02) as shown in (Table2).

PARAMETER	Different grades of OSCC	N	MEAN±SD	p Value
Salivary Uric	Well differentiated OSCC	4	2.9±0.29	
Acid Level	Moderately differentiated OSCC	4	1.3±0.26	0.02
(mg/dl)	Poorly differentiated OSCC	2	1.2±0.25	

Intergroup comparison for salivary uric acid concentration applying post hoc test (Bonferroni test) revealed statistically significant results (p value less than 0.05) as shown in (Table3).

(I) GROUP	(J) GROUP	MEAN DIFFERENCE(I-J)	p value
Healthy Subjects	Odontogenic cysts	3.1	<0.001
	Oral squamous cell carcinoma	4.5	<0.001
Odontogenic cysts	Healthy Subjects	-3.1	<0.001
	Oral squamous cell carcinoma	1.4	0.004
Oral squamous cell carcinoma	Healthy Subjects	-4.5	<0.001
	Odontogenic cysts	-1.4	0.004

DISCUSSION

Oxidative stress is a general term used to describe the steady state level of oxidative damage in a cell, tissue or, organ caused by ROS. This damage can affect a specific molecule or, an organism as a whole.^[14] Most ROS are generated from endogenous sources as byproducts of normal and essential metabolic reactions, such as energy generation from mitochondria or, detoxification reactions that involve the hepatic microsomal enzyme system. Exogenous sources include exposure to cigarette smoke, environmental pollutants such as emission from automobiles and industries, excessive consumption of alcohol, asbestos, exposure to ionizing radiation, and the plethora of bacterial, fungal and viral infections.^[10,15]

The determinants of oxidative stress are regulated by an individual's unique hereditary factors as well as environment and characteristic lifestyle. Several other factors put forth altering uric acid concentration are periodontal disease, recurrent aphthous ulcers, premalignant lesion, antioxidant medications, systemic disease, chronic alcoholics and smokers^[1] which were put into consideration and excluded from the present study.

The effect of inflammation on the cystic epithelial lining and connective tissue stroma has been previously investigated and has shown changes in the morphology and proliferative activity of the epithelium and collagen structure.^[16]

Inflammation begins with a reaction to an irritant or infection leading to recruitment of mast cells and leukocytes to the damaged site. Associated with these processes are the release of free radicals (ROS and RNS) from leukocytes including activated macrophages. This can damage healthy epithelial and stromal tissues, activate a process called lipid peroxidation and arachidonic acid cascade. Increase in free radical formation and decrease in antioxidant defense capacity leads to oxidative stress which can be a prognostic indicator of malignant transformation of odontogenic cyst.^[17]

Several recent studies have found a direct influence of inflammation in the cyst on epithelial cells, either through direct adhesion of inflammatory cells or through an indirect response of chemokines produced by inflammatory cells.^[16,18]

In a recent study by Giebultowicz et al 2011, observed the lowered uric acid concentration in the groups suffering from both odontogenic cysts and oral cavity cancer and suggested that these groups can be exposed to oxidative stress and related risk of DNA damage which might partially explain the mechanism involved in malignant induction in odontogenic cysts.^[1]

In the present study, the salivary uric acid level estimated using 'URIC ACID ROCHE' ASSAY by semi automatic analyser significantly lowered from 6.4mg/dl in healthy control to 3.3mg/dl in odontogenic cyst to 1.8 mg/dl in oral squamous cell carcinoma, which was in accordance with study by (Giebultowicz et al 2011). The lowered level of salivary uric acid concentration can be considered as an important mechanism by which toxic effects of free radicals can initiate malignant transformation of cyst.

TABLE 1: - Overall comparison for salivary uric acid concentration between Healthy Subjects, Odontogenic cyst and Oral Cavity cancer

PARAMETER	GROUPS	N	MEAN±SD	p Value
SALIVARY URIC ACID(mg/dl)	Healthy Subjects	10	6.4± 0.82	0.02
	Odontogenic cysts	10	3.3± 1.14	
	Oral squamous cell carcinoma	10	1.8±0.80	

Analysis of Variance Test. p value <0.05 significant.

Table 2: Comparison for salivary uric acid concentration in different grades of OSCC

PARAMETER	Different grades of OSCC	N	MEAN±SD	pValue
Salivary Uric Acid Level (mg/dl)	Well differentiated OSCC	4	2.9±0.29	0.02
	Moderately differentiated OSCC	4	1.3±0.26	
	Poorly differentiated OSCC	2	1.2±0.25	

Analysis Of Variance, p value less than 0.05

Table 3: Intergroup comparison for salivary uric acid concentration

(I) GROUP	(J) GROUP	MEAN DIFFERENCE(I-J)	p value
Healthy Subjects	Odontogenic cysts	3.1	<0.001
	Oral squamous cell carcinoma	4.5	<0.001
Odontogenic cysts	Healthy Subjects	-3.1	<0.001
	Oral squamous cell carcinoma	1.4	0.004
Oral squamous cell carcinoma	Healthy Subjects	-4.5	<0.001
	Odontogenic cysts	-1.4	0.004

Post hoc test- Bonferroni test, p value at the level of 0.05

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