Analysis of Salivary Antioxidant Levels in Different Clinical Staging and Histological Grading of Oral Squamous Cell Carcinoma: Noninvasive Technique in Dentistry

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ABSTRACT

Objective: To estimate and Compare of salivary antioxidant level {Uric acid (UA), Glutathione S Transferase (GST) and Superoxide dismutase (SOD)} between healthy control and study group (oral squamous cell carcinoma patients).Further comparison of sub division of study group on the basis of clinical staging and histological grading.

Materials and Methods: The study group consists of 50 cases of squamous cell carcinoma and 50 healthy patients. These parameters were estimated by spectrophotometer. The biochemical values of

this study were subjected to statistical analysis i.e. Independent t-test, ANOVA and Tukey test.

Result: UA suggested statistically significant changes in saliva of clinical staging and histological grading of oral squamous cell carcinoma (SCC) patients. Salivary SOD level between well to poorly differentiated SCC showed a progressive increase although it is not statistically significant.

Conclusion: Salivary analysis of antioxidant is simple, non-invasive technique which may be useful as diagnostic, prognostic and therapeutic marker.

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the sixth most common human cancer [1]. The aetiology of oral cancer is multifactorial [2]. In head and neck carcinoma, the treatment and prognosis is usually predicted based on TNM clinical staging and histological grading [3].

The release of free radicals i.e. reactive oxygen species cause loss of salivary antioxidant capacity lead development of oral cancer in many tobacco chewers and smokers [4,5]. Antioxidants, on the other hand have a protective role by scavenging the free radicals [6].

The present study is undertaken to correlate salivary antioxidant levels in different clinical staging and histological grading of OSCC.

MATERIALS AND METHODS

The study and control group comprised of 50 patients each. The study group was further divided into two sub groups based on clinical staging and histological grading. In our pre-active oxygen speciespective study conducted between time period of two years between 2010-2012, we have aimed to achieve following objectives as follow:

1. Comparison of biochemical parameters i.e. SOD, UA & GST of study group patients to control group patients.

2. Comparison of biochemical parameters i.r.t. clinical staging of OSCC of study group.

3. Comparison of biochemical parameters i.r.t. histological grading of OSCC of study group.

   The protocol was reviewed by the institutional review board (IRB), was in compliance with the Helsinki Declaration and that each subject in the project signed a detailed informed consent form.

Inclusion criteria: Patients clinically diagnosed as having oral cancer with confirmed histological findings. The age group was kept under 40-80 year.

Exclusion criteria: Subjects with any local and systemic infections/ illness, oral antioxidant supplements/ medications and with incomplete clinical histopathological details.

We have kept same criteria like Woolgar and scott's histologic grading in our preactive oxygen speciespective study and was classified as either well, moderate, or poorly differentiated [7]. Before collecting the saliva, the subjects were instructed to rinse their mouth with water. The saliva was collected by placing a cotton roll beneath the tongue till it gets soaked. Collected clear saliva was centrifuged at 4000 rpm for 10min & the supernatant was collected for the estimation of Uric acid (UA), Glutathione S Transferase (GST) & Superoxide dismutase (SOD). These parameters were estimated by spectrophotometer. The biochemical values of this study were subjected to statistical analysis i.e. Independent T-test, ANOVA and Tukey test. The parameters used in this study are salivary uric acid, SOD and GST. These parameters were estimated by spectrophotometer.

Determination of Uric Acid Concentration

Salivary and plasma uric acid concentration was measured by Uricase-PAP methodology.

Determination of SOD

The SOD activity was measured according to Beauchamp and Fridovich. SOD activity depends on the capacity of the enzyme to inhibit the reduction of nitroblue tetrazolium (NBT) by superoxide, which is generated by the reaction of photo reduced riboflavin and oxygen [8].

Determination of GST

The GST activity was measured by the method of Paglia and Valentine as modified by Lawrence and Burk. Specific activity was calculated as micromole NADPH consumed per minute per milligram protein (U/mg protein) using an appropriate molar absorption coefficient [9].

RESULTS

[Table/Fig-1a-c] showed data pertaining to our study. The one subgroup (clinical staging) of study group comprised of 50 cases of OSCC out of which 5 cases were of stage I, 5 cases were of stage II, 10 cases were of stage III and 30 cases were of stage IV. The other subgroup (based on histological grading) of study group comprised of 50 cases of OSCC out of which 26 cases were of well differentiated squamous cell carcinoma, 14 cases were of moderately differentiated squamous cell carcinoma and 10 cases were of poorly differentiated squamous cell carcinoma. The biochemical values obtained in the study were subjected to statistical analysis via student t-test, ANOVA and tukey test. [Table/Fig-2] showed that mean of salivary UA, GST & SOD in OSCC patients were statistically less (very highly significant) compare to the healthy control patients at p<.001. [Table/Fig-3] showed GST and

SOD level did not show a statistical significant difference between the clinical staging except for the uric acid (UA) level which showed progressive decrease from stage I to stage IV. However, it was not statistical significant decrease.

[Table/Fig-4] showed statistically significant difference at p=0.05 between intergroup comparison of mean of uric acid in different histological grades of OSCC. [Table/Fig-5] showed Tukey test for UA showed statistically significant difference at p=0.05 between well to moderate and moderate to poorly differentiated squamous cell carcinoma. However, Tukey test for GST showed statistically significant decrease from moderate to poorly differentiated squamous cell carcinoma.

DISCUSSION

OSCC is the sixth most common human cancer, with an increasing

S.No.	AGE/SEX	Grade	Staging	SOD U/mg §	GST in mg/ml ;	UA in mg/dl**
1	52/M	Poor ‡	T2N0M0 (I)	0.011	0.112	1.176
2	45/M	Mod †	T2N0M0 (II)	0.068	0.32	1.642
3	55/M	Well *	T4N2M0 (IV)	0.112	1.08	2.482
4	60/M	Mod	T2N1M0 (III)	0.017	1.23	1.621
5	65/M	Well	T2N2CM0 (IV)	0.007	0.286	2.248
6	58/M	Well	T3N2cMX (IV)	0.014	0.222	2.562
7	48/M	Poor	T4N2cMX (IV)	0.008	0.26	1.168
8	49/M	Poor	T4N2cMX (IV)	0.077	0.165	1.174
9	54/M	Well	T4N2cMX (IV)	0.114	1.05	2.621
10	57/M	Well	T4N2cMX (IV)	0.003	0.575	2.884
11	45/F	Well	T4N2cMX (IV)	0.006	0.422	2.48
12	50/F	Mod	T4N2BMX (IV)	0.003	0.432	1.621
13	56/M	Well	T4N2cMX (IV)	0.018	0.387	3.024
14	60/M	Mod	T2N2bM0 (IV)	0.074	0.32	1.492
15	50/F	Poor	T4N2BMX (IV)	0.021	0.129	1.176
16	58/F	Poor	T3N1MX (III)	0.006	0.168	1.169
17	65/F	Well	T4N2BMX (IV)	0.002	0.289	2.983
18	53/M	Well	T1N0M0 (I)	0.024	0.478	1.994
19	48/M	Well	T2N0M0 (II)	0.018	0.312	3.021
20	45/M	Mod	T2N0M0 (II)	0.037	1.47	1.742

[Table/Fig-1a]: Shows level of salivary antioxidant levels (SOD, GST and UA) in OSCC patients

Sl.No.	AGE/SEX	Grade	Staging	SOD inU/mg §	GST in mg/ml ;	UA in mg/dl**
21	42/M	Well *	T2N2bM0 (IV)	0.018	0.812	2.321
22	50/M	Well	T2N1M0 (III)	0.012	0.672	2.832
23	70/F	Well	T2N1M0 (III)	0.024	0.478	3.08
24	49/F	Well	T4N2CM0(IV)	0.124	0.242	1.982
25	45/F	Well	T3N2M0 (IV)	0.0034	0.296	2.981
26	58/M	Well	T1NN0M0 (I)	0.007	0.674	2.002
27	54/M	Well	T2N0M0 (III)	0.009	0.286	1.989
28	62/M	Mod †	T2N1M0 (III)	0.034	0.207	1.402
29	68/F	Well	T2N0M0 (II)	0.025	0.212	2.088
30	49/M	Poor ‡	T4N1MX (IV)	0.032	0.378	1.082
31	46/M	Poor	T4N1M0 (IV)	0.016	0.227	0.0621
32	58/M	Mod	T4N2bMX (IV)	0.01	0.794	1.762
33	74/M	Mod	T4N2MX (IV)	0.007	0.359	2.001
34	62/F	Mod	T2N2CM0 (IV)	0.024	0.437	1.289
35	65/M	Well	T4N2bM0 (IV)	0.008	0.64	3.573
36	63/F	Poor	T4N1M0 (IV)	0.027	0.316	1.112
37	58/F	Well	T1N0M0 (I)	0.039	0.718	3.173
38	65/M	Well	T1N1M0 (III)	0.024	0.478	3.01
39	68/M	Mod	T4N2bM0 (IV)	0.021	1.129	1.498

[Table/Fig-1b]: Shows level of salivary antioxidant levels (SOD, GST and UA) in OSCC patients

S. No.	AGE/SEX	Grade	Staging	SOD inU/mg §	GST in mg/ml ¦	UA in mg/dl**
40	42/F	Well*	T4N1M0 (IV)	0.008	0.369	4.232
41	72/F	Well	T4N1M0 (IV)	0.012	0.312	2.561
42	68/M	Poor	T3N1M0 (IV)	0.014	1.075	0.0291
43	78/M	Well	T4N1M0 (IV)	0.018	0.387	2.261
44	65/M	Mod †	T2N1M0 (III)	0.021	0.265	1.782
45	45/M	Poor †-	T4N2CM0 (IV)	0.077	0.387	0.0281
46	58/M	Well	T2N1M0 (I)	0.0051	0.32	5.17
47	62/M	Mod	T2NXM0 (II)	0.037	0.105	2.981
48	59/M	Well	T3N1M0 (III)	0.018	0.388	2.98
49	70/M	Mod	T3N1M0 (III)	0.021	1.129	1.982
50	63/M	Mod	T4N1M0 (IV)	0.009	0.674	1.456

[Table/Fig-1c]: Shows level of salivary antioxidant levels (SOD, GST and UA) in OSCC patients.i
*Well differentiated squamous cell carcinoma, †- Moderately differentiated squamous cell carcinoma, ‡ poorly differentiated squamous cell carcinoma, §-Superoxide dismutase, ¦-Glutathione S- Transferase, ** Uric acid

GROUP	n	Mean	Std Deviation	t
UA ‡ Control	50	5.9675	1.07319	-18.685
Case	50	2.0996	.99535	P<.001vhs
GST † Control	50	.1861	.07226	6.40500
Case	50	.4895	.32704	P<0.01vhs
SOD* Control	50	.9911	1.20974	-5.63400
Case	50	.0269	.02937	P<0.01vhs

[Table/Fig-2]: Shows the comparison of mean of salivary UA, GST & SOD in OSCC patients and healthy control group.
*-Superoxide Dismutase, †- Glutathione S- Transferase, ‡- Uric acid

Group	n	Mean	Std. deviation	f	P
Case UA‡ Stage I	5	2.703	1.55184		
Stage II	5	2.2948	0.66572	0.061	.941
Stage III	10	2.1847	.72555		
Stage IV	30	1.9382	1.0092		
Case GST† Stage I	5	.46040	.251688		
Stage II	5	.48380	.558189	.067	.977
Stage III	10	.53010	.374117		
Stage IV	30	.48170	.291174		
Case SOD * Stage I	5	.01722	.014233		
Stage II	5	.03700	.019144	.715	.548
Stage III	10	.01860	.008195		
Stage IV	30	.02958	.035968		

[Table/Fig-3]: Shows the comparison of UA, GST, and SOD mean in clinical staging of OSCC patients.
*-Superoxide Dismutase, †- Glutathione S- Transferase, ‡- Uric acid

	n	mean	Std Deviation	f	P
Uric acid Well	26	2.635	0.68372		
Moderate	14	1.73364	1.12363	1.395	0.000
Poor	10	0.8176	1.50258		
GST Well	26	.47635	.238721		
Moderate	14	.63364	.440465	2.907	0.065
Poor	10	.32170	.282250		
SOD Well	26	.02587	.034579		
Moderate	14	.02736	.021382	.039	.961
Poor	10	.02890	.026610		

[Table/Fig-4]: Shows the comparison of UA, GST, and SOD mean in histological grades of OSCC patients

Group (J) GRADE	Dependent Variable	(I)GRADE	Mean Difference(I-J)	P
Case		Well Moderate	0.90136	0.001
	UA ‡	Poor	1.8174	0.001
		Moderate Poor	0.91604	0.002
Case		Well Moderate	-.15730	.297
	GST†	Poor	.15465	.392
		Moderate Poor	.31194	.005 sig
Case		Well Moderate	-.00149	.988
	SOD*	Poor	-.00303	.960
		Moderate Poor	-.00154	.992

[Table/Fig-5]: Tukey test shows the intra group comparison of histological grades of OSCC patients
*-Superoxide   Dismutase, †- Glutathione S- Transferase, ‡- Uric acid

incidence in younger generation and a five year mortality rate of approximately 50% [10]. TNM clinical staging and histological grading which depicts the immunological relationship between tumour and host, predicting lesion's behavior through patient's response. MA et al., recently demonstrated free radicals such as reactive oxygen species and reactive nitrate species, which induce nitrative and oxidative stress, are main inducers of OSCC [11].

The OSCC-inducing reactive oxygen species and reactive nitrate species originate mainly from smoking, alcohol, food, drink, and/or various other volatile sources, which go to oral cavity causing deleterious effects. Our oral cavity is destined with an unconventional salivary antioxidant system that also contains anti-nitrate and oxygen species amine inhibitory agents. This salivary antioxidant coordination is based on enzymatic and non-enzymatic components including peroxidase and SOD enzymes as well as UA molecules [12]. It also includes another crucial anticancer salivary enzyme, GST, which catalyzes glutathione conjugation to the carcinogen electrophilic epoxide intermediates to protect against DNA damage [13].

Saliva plays a key role in OSCC pathogenesis was also supported by Wu et al., [14]. Diet derived availability of various antioxidants either directly or indirectly correlate with the protection against oxidative stress [15]. In the present study the levels of UA in saliva of patients with squamous cell carcinoma was significantly less than healthy control group (p<.005). Salivary UA accounts for approximately 70% of the total salivary antioxidant capacity [16].Tsuchiya et al., also presented that UA concentration drastically goes down even with the single consumption of cigarette [17]. The Maria Greabu et al., study showed that exposure to the gas phase of cigarette smokers (CS) caused a statistically significant fall down in salivary uric acid (p < 0.05) [18].

Changes in the salivary antioxidant enzymes suggest that saliva may be appropriate marker for the prognosis of oral diseases compare to our conventional invasive serum antioxidant enzyme [18]. It is well understood that superoxide ion (O_2^-) is first to be formed in the chain production of free radicals. Initially, SOD inactivates O_2^- by transforming it into hydrogen peroxide (H_2O_2) and further action by catalase and peroxidases into dioxygen (O_2) and water (H_2O) [19].

Decrease in SOD levels in the patients of OSCC correlates with the study by Manoj sharma et al., which says decrease in SOD activity in target cells of OSCC which lead to formation of O_2^- and H_2O_2, a highly diffusible and potent oxidizing radical capable of traversing membranes, lead to deleterious effects at sites far from the tumour [20].

And also, Aashita Gupta et al., showed reduction of SOD activity may also be due to increased endogenous production of reactive oxygen species as demonstrated by elevated Malondialdehyde (MDA) levels [21].

In the present study the levels of GST in saliva of patients(study

group) with squamous cell carcinoma showed very high statistically significant increase compare to control group at $p<.001$. GST is vital antioxidant present in cells. Rapid GST synthesis in tumour cells is associated with high rates of cell proliferation, while GSH depletion is sufficient to sensitize cancer cells to the cytotoxic effects of oxidative and nitrative stress and make them more defenseless to the effects of anticancer drugs or the genes that promote apoptosis [22].

All the parameters which have been taken in our study are important to know progression, prognosis and treatment outcome through merely based on our simple salivary test. These tests are much easier and painless compare to our routine blood test, which sometime might be very cumbersome for patient and doctors as well. Sensitivity and specificity are also near to our conventional serum test. According to Natheer H Al-Rawi et al., was based to correlate serum and salivary antioxidants to differentiate ischemic stroke patients from otherwise healthy individuals [23]. Serum UA gave value of accuracy 89.3% and sensitive by 96%, whereas, salivary UA was accurate by 89.3% and sensitive by 92% only. Serum and salivary GSH share same critical value of accuracy (80%) and (86-90%) sensitivity. Salivary critical value of SOD gave 89.3% accuracy and 100% specificity in comparison with serum SOD with only 80% accuracy. There is no harm in performing these or may be adjuvant to our serum test. So, we need to focus and do more number of studies to bring about changes in our routine for the benefit of patients.

Henceforth, we can perform this upcoming non-invasive technique over invasive serum analysis of antioxidant for the diagnostic and therapeutic purpose for the benefit of OSCC patients and physicians too.

CONCLUSION

The present study done on antioxidants and its relation to OSCC suggest that antioxidant may play a major role in prevention of oral cancer and its utilization as diagnostic and prognostic marker. Antioxidants are necessary for our health but we do not know the exact dose and the way how to supplement it, so further research is required to know more about antioxidants.

1. Decreased salivary UA and SOD in oral squamous cell carcinoma than normal healthy patients, except for GST levels which showed statistically significant increased level compare to healthy control group.

2. Among histological grading of squamous cell carcinoma salivary UA showed progressively decrease from well to moderate and moderate to poorly differentiated squamous cell carcinoma with highly statistical significant increase (at $p<0.05$).

3. SOD salivary level between well to poor showed a progressive increase although it is not statistically significant.

4. UA level which showed progressive decrease from stage I to stage IV clinical staging.

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