ORIGINAL ARTICLE

Evaluation of potential salivary acetaldehyde production from ethanol in oral cancer patients and healthy subjects

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Abstract

Background: Acetaldehyde has been implicated as a major factor in oral carcinogenesis associated with alcohol consumption. In this study, saliva samples from oral cancer patients and healthy individuals were incubated in vitro with ethanol in order to investigate factors which can influence salivary acetaldehyde production.

Materials and Methods: A total of 66 individuals (40 males and 26 females, mean age 52 years) participated in the study. Participants were classified into three groups: Group 1 (oral cancer patients [n = 20]); Group 2 (poor dental health status [n = 25]) and Group 3 (good dental health status [n=21]). Every patient chewed a 1g piece of paraffin chewing gum for 1 minute then saliva samples were collected from all individuals. After in vitro incubation of the samples with ethanol, the levels of salivary acetaldehyde production was measured by head space gas chromatography. Kruskal-Wallis and Mann-Whitney tests and Spearman's Correlations analysis were performed for statistical analyses.

Results: The salivary acetaldehyde production was significantly higher ($p < 0.0001$) in both group 1 and group 2 when compared to group 3. However, there was no significant difference between group 1 and group 2. Poor dental health status, infrequent oral hygiene habits and dental visits, smoking and presence of a dental prosthesis were significant parameters for increased levels of salivary acetaldehyde production from alcohol. The evaluation of salivary acetaldehyde production after in vitro incubation with ethanol may be useful for early detection of oral cancer.

Conclusion: According to the results of this study, the significantly higher levels of salivary acetaldehyde production in oral cancer patients and individuals with poor dental health status may suggest a possible link between increased salivary acetaldehyde production and oral cancer. Improved oral hygiene can effectively decrease the level of salivary acetaldehyde production in oral cavity. Hippokratia 2014; 18 (3): 269-274.

Keywords: Oral cancer, salivary acetaldehyde production, oral hygiene, dental health status

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Introduction

Excessive alcohol consumption is a significant risk factor for squamous cell carcinoma in the upper aeodigestive tract (UADT)[1-7]. There is increasing evidence that a major part of the tumour-promoting action of alcohol might be mediated via its first, toxic and carcinogenic metabolite acetaldehyde. Recent biochemical and epidemiological findings strongly suggest that acetaldehyde, the first metabolite of ethanol, plays an important role in alcohol-related carcinogenesis in the UADT consisting oral cavity, pharynx, larynx and esophagus[5-8]. Acetaldehyde (CH_3 CHO, CAS # 75-07-0) is a metabolite of ethanol which comprises in the human body after the consumption of alcoholic beverages[9-11]. Intracellular formation of acetaldehyde from ethanol is produced in the epithelial cells by mucosal alcohol dehydrogenase (ADH), however much higher levels derive from microbial oxidation of ethanol by the oral microflora. The enzymatic conversion of ethanol produced by the physiological oral microflora may lead to accumulation of increased levels of the carcinogenic intermediate acetaldehyde[2,12,13]. Most epidemiological studies revealed that the indicators of poor dental hygiene such as tooth loss, poor dentition and infrequent practice of oral hygiene habits are only weak risk factors for oral cancer. Nevertheless, there is evidence to suggest that the higher incidence of poor dental status may contribute to the carcinogenicity of alcohol in individuals consuming high levels of alcohol[12]. In previous studies[2,12], it has been demonstrated that the production acetaldehyde from ethanol increases in smoking, heavy

drinking and poor dental status. However, the factors influencing acetaldehyde production after incubation of saliva in ethanol are unclear in oral cancer patients compared to healthy individuals. The aim of this study was to evaluate the factors which can influence the production of acetaldehyde in saliva after in vitro ethanol incubation of salivary samples obtained from oral cancer patients and healthy individuals.

Materials and Methods

A total of 66 individuals (40 males and 26 females; mean age 52 years, age range 32 - 80 years) participated in this study. Healthy subjects were selected from among the non-drug user and systemically healthy volunteers who referred to the Department of Oral and Maxillofacial Surgery, Faculty of Dentistry, Istanbul University, Istanbul, Turkey. Oral cancer patients were selected from among the patients who referred to the Institute of Oncology, Istanbul University, Istanbul, Turkey. These patients had been diagnosed, as confirmed histopathologically, with squamous cell carcinoma of tongue and were to receive radiotherapy. Academic ethical approval was obtained from Istanbul University. The study followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants. Exclusion criteria were as follows: treatment with oral antiseptic or antibiotics in the past month; food or fluid intake, smoking or tooth brushing in the preceding 90 min; recent alcohol intake or quantifiable amount of alcohol in the saliva determined by head space gas chromatography. Each volunteer filled in a structured questionnaire. Information regarding age, gender, smoking, alcohol consumption, diet, oral hygiene habits and other characteristics were elicited. Smoking status was ranked as daily average number of cigarettes, pipes or cigars smoked within the past 30 days. Daily tobacco consumption was calculated as cigarettes smoked per day (1 cigar = 3 cigarettes, 1 pipe = 5 cigarettes). Ex- smokers who stopped smoking for ≥5 years were ranked as non smokers. Ex- smokers who stopped smoking only recently were excluded from the study. Those smoking up to 5 cigarettes and 6 cigarettes or more were ranked as moderate smokers and heavy smokers, respectively. Alcohol status was estimated as the average amount of alcoholic beverages (12 g of pure alcohol) for every drinking day during the past 30 days, and as the frequency of alcohol intake per week. Based on these data, the average amount of consumed alcohol was calculated as gram pure ethanol/day. Volunteers were ranked as non-drinkers (<1 gram/day), moderate drinkers (1-30 gram/day) and heavy drinkers (>30 gram/day). Every participant underwent a detailed dental examination by an experienced dentist. Diseased tooth surface (DS) and diseased missing filled teeth (DMF) indices were recorded according to the World Health Organization's recommendations[14]. Patients were classified as follows: a DS score higher than 4, regardless of the DMF index higher than 2, if DMF was at least 30 led to a classification of a dental status "poor" (n=25). Patients with a DS score lower than 3 and a DMF index of maximum 21 were ranked as having a "good" dental heath status (n=21). In overall, there were 20 patients with oral cancer (group 1), 25 individuals with poor dental health status (group 2) and 21 individuals with good dental health status (group 3). Every individual was asked to chew a 1g piece of paraffin gum (Intergum Gıda San. Tic. AS, Istanbul, Turkey) for 2 minutes and then a saliva samples were collected between 9 a.m. and noon. The saliva samples were immediately stored at -70°C. The production of acetaldehyde from ethanol in each sample was measured by head space gas chromatography method as described by Homann et al[1]. Head space gas chromatographic analysis were performed under the following conditions: Termo Quest Trace GC 200 Series/ column 60/80 Carbopack B/5% Carbowax 20M, 2 mx1/8 inch (Supelco, Bellefonte, PA); oven temperature 85° C; transfer line and detector temperature 200° C; carrier gas flow rate (N_2) 20 ml/min. Before the analysis, the frozen saliva samples were thawed and preheated up to 37°C. A 400 µl of saliva sample was transferred to a gas chromatograph vial. Then a 50 µl of potassium phosphate buffer containing ethanol was added into each vial in which the final concentration was 22 mM. Vials were immediately closed tightly and incubated for 90 minutes. The reaction was stopped by injecting 50 µl of 6 M perchloric acid through the rubber septum of the vial. All samples were measured as triplicates. For each saliva sample one analysis was carried out by concomitantly adding 50 µl perchloric acid and an ethanol/potassium phosphate buffer mixture, before addition of 400 µl of saliva. These control assays for baseline and artifactual acetaldehyde production were incubated for 90 min and revealed values were subtracted from the acetaldehyde levels after 90 min incubation with ethanol. In the statistical analyses, all values are reported in $\overline{X}$ (mean) ± SD (standard deviation) and median (min-max), if not otherwise mentioned. Mann Whitney U test was performed to reveal the differences between two groups. Kruskal-Wallis test followed by multiple comparison procedure was performed to differentiate among three groups. Spearman's Correlations Analysis was carried out to determine the correlations of the acetaldehyde production levels from ethanol. Wilcoxon test was carried out to reveal the differences between two paired groups. All reported p-values were derived from two-sided tests. A p-value less than 0.05 was considered statistically significant. Statistical analyses were achieved by a software (MedCalc, Belgium, version 11.6.0.0.).

Results

The gender distribution of individuals were similar among all three groups (x^2=1.99; p=0.158). The mean age was 52.36 ± 10.77 (median 51, min 32 - max 80) years. The characteristics of the study population and the effects of different variables on the salivary acetaldehyde production were shown in Table 1. The level of acetaldehyde production between group1 (oral cancer patients) and group 2 (poor dental health status) did not differ significantly. However, the level of acetaldehyde production was significantly higher in group1 (oral can-

Table 1: Salivary Acetaldehyde Production Analysis (μmol/L).

	n	$\overline{X}$ mean ± SD; median (min-max)	Kruskal-Wallis test	
			x^2	p value
Group				
oral cancer[a]	20	140.1 ± 15.7; 145.8 (193.5-157.4)		
poor dental status[a]	25	147.4 ± 15.4; 147.8 (119.9-174.0)		
good dental status[b]	21	53.6 ± 24.5; 57.8 (14.4-99.4)	43.1	0.0001***
Smoking status				
non-smoker [a]	28	90.97 ± 49.2; 88.9 (44.4-158.2)		
moderate[b]	24	126.91 ± 42.8; 146.2 (35.9-174.0)		
heavy[b]	14	144.26 ± 10.8; 147.9 (118.2-157.4)	14.19	0.001**
Tooth brushing				
rare[a]	50	125.6 ± 41.2; 143.3 (14.6-174.0)		
1-2[a]	5	119.67 ± 51.1; 122.1 (35.9-168.2)		
3+ [b]	11	66.8 ± 39.3; 64.7 (14.4-140.2)	13.19	0.001**
Dental visit				
1 per year [a]	13	65.8 ± 39.2; 64.7 (14.4-149.3)		
1 every 2 years[a]	6	47.1 ± 19.7; 46.9 (24.1-70.2)		
rare[b]	47	137.8 ± 28.2; 145.8 (30.3-174.0)	30.05	0.0001***
Dining frequency (daily)				
1-2	13	89.47 ± 50.4; 70.2 (14.4-165.5)		
2-3	41	119.21 ± 46.5; 140.7 (14.6-174.0)		
4+	12	130.17 ± 32.2; 140.7 (51.4-173.4)	4.07	0.131

		$\overline{X}$ mean ± SD; median (min-max)	Mann-Whitney U test	
			z	p value
Alcohol intake				
-	56	114.8 ± 48.8; 139.9 (14.4-174.0)	0.32	0.748
+	10	118.6 ± 32.3; 121.8 (64.7-157.4)		
Prosthesis				
-	47	103.8 ± 50.2; 119.9 (14.4-174.0)	2.49	0.013*
+	19	143.8 ± 13.1; 145.8 (11.8-173.4)		
Mouthwash use				
-	57	117.1 ± 46.0; 138.5 (14.6-174.0)	1.09	0.274
+	9	104.2 ± 50.8; 122.1 (14.4-149.2)		
Gender				
Female	23	81.3 ± 52.5; 65.1(14.4-158.2)		
Male	43	133.5 ± 30.4;145.8 (61.0-174.0)	3.80	0.0001***
Age				
32-55	42	101.5 ± 51.5;120.3 (14.4-173.4)		
56+	24	139.6 ± 20.5; 146.6 (84.4-174.4)	2.49	0.013*

$\overline{X}$: mean, SD: standard deviation, *p< 0.05, **p<0.01, ***p<0.001.

cer patients) and group 2 (poor dental health status) compared to group 3 (good dental health status) (p=0.0001). The level of salivary acetaldehyde production was significantly higher in moderate and heavy smokers than non-smokers (p=0.001). On the other hand, there was no significant difference between moderate and heavy smokers. The acetaldehyde production was significantly lower in individuals tooth brushing three times or more per day (p=0.001). The acetaldehyde production in saliva was significantly higher in males (p=0.0001) and in the elderly (age range 56-80 years) (p=0.013). The salivary acetaldehyde production level in participants making dental visits annually or biyearly was lower than the participants making rare dental visits (p=0.0001). In this study, the count of alcohol consumers were relatively low (n=10). There were 6 patients in oral cancer group, two person in poor dental health status group and, other two being in good dental health status group. In addition, none of the participants consumed alcohol over 30 g per day. Since there was no oral cancer patient consuming heavy alcohol (over 30 g per day), the statistical comparison was only made between alcohol users and non-users. There was no significant difference (p=0.74) between alcohol users and non-users with regard to salivary acetaldehyde production. In general, the level of acetaldehyde production in patients wearing a dental prosthesis was significantly higher than those who were not wearing a dental prosthesis (z=2.49 and p= 0.013). There was no significant difference in the level of acetaldehyde production between denture wearers and non-wearers in patients older than 56 years of age. The daily eating habits and the use of alcohol-free mouthwash or alcohol did not seem to significantly alter the salivary acetaldehyde production. The acetaldehyde correlations analysis is given in Table 2. According to the results of this study, acetaldehyde level was positively correlated with age and the number of missing teeth (Spearman's rho= 0.44 and p=0.0001). Univariate analyses for the different variables revealed that oral cancer, poor dental health status, heavy smoking, wearing a prosthesis, rare dental visits and infrequent tooth brushing were statistically significant parameters which led to an increase in salivary acetaldehyde production from ethanol.

Table 2: Salivary Acetaldehyde Production Correlation Analysis.

	Acetaldehyde levels	
n: 66	**rho**	**p**
DS	0.44	0.0001
D	0.42	0.0001
M	0.59	0.0001
F	0.33	0.007
Age	0.44	0.0001

DS: diseased tooth surface, DMF: diseased (D) missing (M) filled (F) teeth, rho: Spearman's rho correlation coefficient.

Discussion

Smoking, heavy drinking and poor dental status are well-known risk factors for UADT cancers and they are also the strongest factors for increased salivary acetaldehyde production. Acetaldehyde accumulates in oral cavity after alcohol intake and is responsible for an increased risk factor for alcohol-related squamous cell carcinoma in UADT[2,5-7]. On the other hand, aldehyde dehydrogenase (ALDH) catalyzes the oxidation of acetaldehyde to non-toxic acetic acid. In humans, there are multiple forms of ALDH, and the enzyme encoded by *ALDH2* on chromosome 12 is thought to play a major role in the detoxification of acetaldehyde[10]. In 40-50% of East Asian population, *ALDH2* exhibits low activity due to a single nucleotid polymorphism[9]. Inactive heterozygous *ALDH2* increases the risk of UADT cancer in drinkers[15]. Acetaldehyde has been shown to be toxic, mutagenic and carcinogenic in animal experiments[3], and its minimum mutagenic concentrations estimated between 50 μM and 150 μM [6,7]. Although poor oral hygiene and poor dentition, faulty restorations, sharp teeth and ill-fitting dentures had been implicated in some epidemiological studies[16-18] it is not clear whether confounding by tobacco and alcohol addressed in these studies. While it is likely that chronic irritation from dental factors may facilitate exposure to carcinogens this may act as a co-factor in only high-risk individuals. Oral microorganisms may also be a factor in chronic alcohol users as some microorganisms facilitate the metabolism of ethanol to acetaldehyde in the oral cavity. This may contribute to acetaldehyde formation in the oral environment and acetaldehyde adducted to oral cancer cells among chronic alcoholics was recently demonstrated[19]. There is an obvious relationship between increased oral cancer risk and poor oral hygiene. This association has been observed in studies from United States, various European countries and People's Republic of China. There is no available data from developing countries[12]. The results of our study showed that oral cancer, poor dental health status, smoking, wearing prosthesis, infrequent tooth brushing and rare dental visits were statistically significant parameters which increased salivary acetaldehyde production in a Turkish population. Although there were a number of cited studies, only one study analyzed the risk factors for oral cancer patients who were non- tobacco and non-alcohol users. This study showed that poor dental status was not associated with increased cancer risk[20]. In our study, salivary acetaldehyde production was elevated in both oral cancer patients and individuals with poor dental health status. In addition, the level of salivary acetaldehyde production did not differ significantly between these groups. There was also no significant difference in salivary acetaldehyde production between 10 low level alcohol users and 56 non- alcohol users, and there was no participant consuming alcohol over 30 g per day. Only were 9 participants using alcohol-free mouthwash included in the study while one participant using alcohol-containing mouthwash was excluded from the study. Recently, La Vecchia[21] reviewed epidemiological studies published over the last three decades, three stud-

ies reported relative risks and seven reported no consistent association. Regular mouthwash use was suspected to be associated with increased cancer risk[22]. The evaluation in the former studies was made without epidemiological evidence[6,23]. Some epidemiological studies available to examine any associated risks, two showed significant increases[24,25]. Blot et al[22], showed non- significant elevated risks and some studies indicated oral cancer risks among mouthwash users[26-30]. In addition to a lack of a consistent association, a dose response relation has not been established. Lachenmeier et al[31], showed that the use of alcohol-containing mouthwash led to an increase in salivary acetaldehyde and this was a significant risk factor for oral cancer. In our study, we did not find out any statistically significant relation between alcohol-free mouthwash use and salivary acetaldehyde level. However, these findings might simply derive from the fact that use of mouthwashes might merely affect the actual level of oral hygiene or oral health status. Acetaldehyde is a metabolite of ethanol which occurs in the human body after the consumption of alcoholic beverages; additionally present in foods, beverages and industry, as well as environment. Limited epidemiological study points to acetaldehyde as an independent risk factor for cancer during alcohol consumption[11]. Acetaldehyde is cumulative carcinogen in humans and for that reason the ALARA principle (as low as reasonably achievable) to acetaldehyde levels of alcoholic beverages, tobacco smoke and also to other beverages and foods produced by fermentation can be applied[11,32]. Acetaldehyde exposure can be decreased by using a special medical device that slowly release L-cystein[33,34]. Improvement of oral hygiene can also be beneficial for the reduction of acetaldehyde production[32].

Conclusions

Within the limitations of this study, the salivary acetaldehyde production level was significantly higher in oral cancer patients and individuals with poor dental health status when compared to individuals with good dental health status. Our results demonstrated that poor dental health status and infrequent oral hygiene habits could be associated with the pathologic mechanisms of oral cancer risk because of increased salivary acetaldehyde production levels. The results of this study showed that smokers in a Turkish population with poor dental health status, infrequent dental visits and poor brushing habits had high acetaldehyde production levels. They were more prone to oral cancer. The pathologic mechanisms for oral cancer risk due to increased salivary acetaldehyde production levels might be associated with poor dental health status. The results of this study emphasized that the frequent dental visits and improved oral hygiene are necessary in order to prevent oral cancer. The evaluation of salivary acetaldehyde production may be useful for early detection of oral cancer.

Conflict of Interest

Authors have no conflicts of interest to declare.

Acknowledgements

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