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Dr. Dhanraj T
Oral Medicine & Radiologist,
Private Practitioner, Ssv Smile
Kare, No.74 Bhraminal Street
Pondicherry, India

Dr. Vandana S
Reader, Dept. of. Oral Medicine
& Radiology, IGIDS, Bahour,
India

Dr. Swetha Paulose
Senior Lecturer, Dept. of. Oral
Medicine & Radiology, IGIDS,
Bahour, India

Comparison of salivary acetaldehyde level in smokers with and without leukoplakia and its correlation with the histopathological findings

Dr. Dhanraj T, Dr. Vandana s, and Dr. Swetha Paulose

Abstract

Smoking is the predominant etiological factor for the development of potentially malignant disorder and Oral Leukoplakia is the most common potentially malignant disorder seen in the oral mucosa.

Aims and Objectives: To estimate salivary acetaldehyde levels in smokers and compare the acetaldehyde levels between smokers with leukoplakia and smokers without leukoplakia.

Materials and Method: A Total of 126 subjects with the habit of smoking were included in the study. Smokers with leukoplakia Group A (n 63) and without Leukoplakia the Group B (n 63). Unstimulated whole saliva was collected from the cases and controls. Salivary acetaldehyde level was estimated using Gas chromatography mass spectrum.

Results: Smokers with leukoplakia had significantly higher level of acetaldehyde ($155.10 \pm 9.59 \mu\text{l}$) in comparison to the Smokers without leukoplakia ($116 \pm 4.8 \mu\text{l}$). ($P < 0.001$).

Conclusion: Salivary acetaldehyde levels are higher in smokers with oral leukoplakia but do not show a statistically significant correlation with the histopathological grading of Oral Leukoplakia.

Keywords: Carcinogen, oral leukoplakia, smoking tobacco, salivary acetaldehyde

Introduction

Carcinoma and carcinogens are the two most feared odds in the current scenario of medical sciences. Carcinoma is a deadly disease with the poorest prognosis and survival rate. It badly affects the quality of life of the affected individual. It has been proved in many circumstances that carcinogens play a major role in development of carcinomas. Acetaldehyde is one such toxic carcinogen which is synthesized in the liver, where it is rapidly metabolized into acetic acid and water. The synthesized quantity in body is usually considered harmless. Important sources that increase acetaldehyde in human body are alcoholic beverages and tobacco smoke. In fact acetaldehyde is the single most abundant carcinogenic substance in tobacco smoke ^[1].

Carcinoma is the second leading cause for global death. According to WHO around 8.8 million death occurred due to carcinoma in 2015. Globally one in six death is occurring due to carcinoma. In India 20 per 100000 population are affected by oral cancer accounting for 30% of carcinoma. Usage of tobacco in the form of smoking has 5.2 times higher risk for precancerous lesion than chewing tobacco ^[2].

Smoking is a predominant etiological factor of the potentially malignant disorder (Oral leukoplakia). Oral Leukoplakia (OL) is the most common potentially malignant disorder of the oral mucosa. It has been defined as "a predominantly white lesion of the oral mucosa that cannot be characterized as any other definable lesion". Leukoplakia shows characteristic histologic findings such as epithelial hyperplasia and/or hyperkeratosis, with or without epithelial dysplasia or carcinoma ^[3].

Tobacco smoking is identified as one among the common cause for both oral leukoplakia and acetaldehyde (carcinogen) production that affects the human body.

The extent of toxicity by acetaldehyde has been studied by various people in the alcoholics as the acetaldehyde levels have been proved to increase after ingestion of alcohol ^[4].

We intend to take the least considered factor smoking and relating it to acetaldehyde level with a possible thought, smoking alone could be a cause for the increase in the carcinogenic

Correspondence

Dr. Dhanraj T
Oral Medicine & Radiologist,
Private Practitioner, Ssv Smile
Kare, No.74 Bhraminal Street
Pondicherry, India

Substance. Primary risk factor for potentially malignant disorder (Oral leukoplakia) is tobacco smoking. Acetaldehyde (Carcinogen) is readily absorbed in the oral cavity from the smoke produced during smoking. In the literature there is a very sparse evidence associating the carcinogen (Acetaldehyde) with the potentially malignant disorder (Leukoplakia). We in our study are estimating the salivary acetaldehyde in smokers who present with leukoplakia. This helps us in understanding the relationship between smoking and the level of carcinogen in leukoplakia.

An increase in carcinogen is a high risk for malignancy. In our study we estimate the level of acetaldehyde (Carcinogen) from saliva, thereby an early detection of the carcinogen level helps us in preventing a deadly disease (Carcinoma). Saliva is a noninvasive biomarker and early detection helps in preventing a deadly disease which reduces the existing burden of the population.

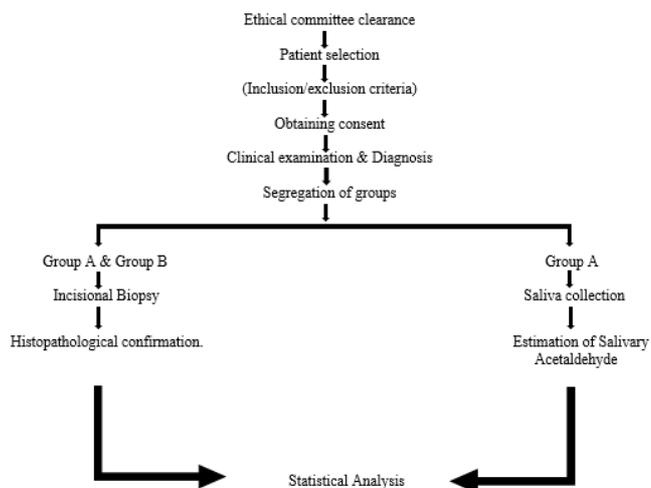
Aims and objectives

AIM: To estimate salivary acetaldehyde levels in smokers with leukoplakia in comparison to smokers without leukoplakia

Objectives

1. Compare the salivary acetaldehyde levels and in the above two groups.
2. Correlate the salivary acetaldehyde levels with histopathological findings of leukoplakia.

Flowchart of study plan



Results and Observation

The study was conducted in 126 patients (n=126) age ranging between 20 to 72years who were smokers. Among them, patients in GROUP A (n=63) had clinically and histopathologically diagnosed Oral leukoplakia while GROUP B (n=63) were smokers without leukoplakia. The salivary samples were collected from 126 subjects, analyzed for salivary acetaldehyde levels using head space gas chromatography.

Age distribution

Group A (Smokers with leukoplakia) consisted of 63 patients, with the age ranging between 20-70 years and an average age of 50.14 years (50.14 ±10.76 years) (Table 1, Figure 1). It was inferred from our study that the peak occurrence of Leukoplakia was between the age group of 51-60 years.

Group B(smokers without leukoplakia consisted of 63 patients, with the age ranging between 20- 70years with an average of 42.66 years (42.66±12.03).The peak occurrence of smokers without leukoplakia were between the age group of 31-40(28.6%).(Table 2, Figure 2).

Distribution of degree of dysplasia in leukoplakia

Histopathological grading of leukoplakia showed 28 cases (44.44%) with hyperkeratosis, 33 cases (52.3%) having mild dysplasia and 2 cases (3.17%) of moderate dysplasia. (Table 3, Figure 3).

Estimation of salivary acetaldehyde in smokers with leukoplakia and smokers without leukoplakia

The salivary acetaldehyde levels from the obtained samples were analyzed with gas chromatography. The mean salivary acetaldehyde value for group A (Smokers with leukoplakia) was 155.10±9.59µl while the Mean salivary acetaldehyde level for group B (Smokers without leukoplakia) was 116±4.8µl. (Table 4, Figure 4). It was evident that the mean salivary acetaldehyde level of group a (Smokers with leukoplakia) was significantly higher when compared to group B (Smokers without leukoplakia).

Comparison of salivary acetaldehyde in smokers with Leukoplakia Asnd smokers without leukoplakia

The mean salivary acetaldehyde value for group A (Smokers with leukoplakia) was 155.10±9.59µl while the mean salivary acetaldehyde level for group B (Smokers without leukoplakia) was 116±4.8µl.The mean difference between the two groups was 38.54 (35.86 – 41.21). The mean difference between the two groups was statistically significant with a p – value <0.0001. (Table 5, Figure 5).

Correlation between histopathological grading and salivary acetalehyde

On comparison between histopathological grading and salivary acetaldehyde level. 28 cases of hyperkeratosis showed a mean salivary acetaldehyde level of 155.5±11.7 µl, 33 cases with mild dysplasia showed a mean salivary acetaldehyde level of 153.8±7.7µl. while 2 cases of moderate dysplasia shows a mean salivary acetaldehyde value of 154±1.1µl. There exists a difference between the groups, yet its statistically insignificant. (p>0.5) (Table 6, Figure 6).

Table 1: Age distribution (Group A)

Age	No. of. patients	Percentage (%)
21-30	4	6.3%
31-40	9	14.3%
41-50	17	27%
51-60	18	28.6%
61-70	15	23.8%

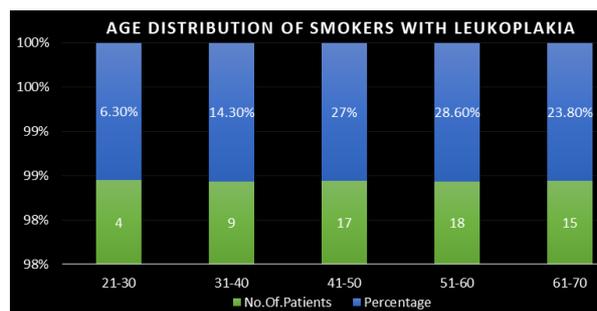


Fig 1: Age distribution (Group A)

Table 2: Age distribution (Group B)

Age	No. Of. Patients	Percentage (%)
21-30	11	17.5%
31-40	18	28.6%
41-50	14	22.2%
51-60	14	22.2%
61-70	6	9.5%

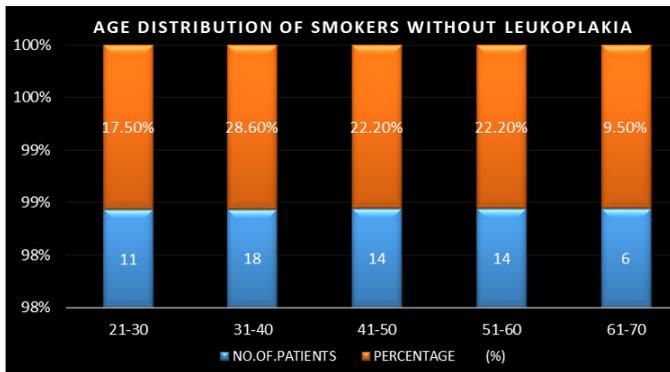


Fig 2: Age distribution (Group B)

Table 3: Distribution of degree of dysplasia in leukoplakia

Histopathological grading	No. of patients	Percentage
Hyperkeratosis	28	44.44%
Mild dysplasia	33	52.3%
Moderate dysplasia	2	3.17%

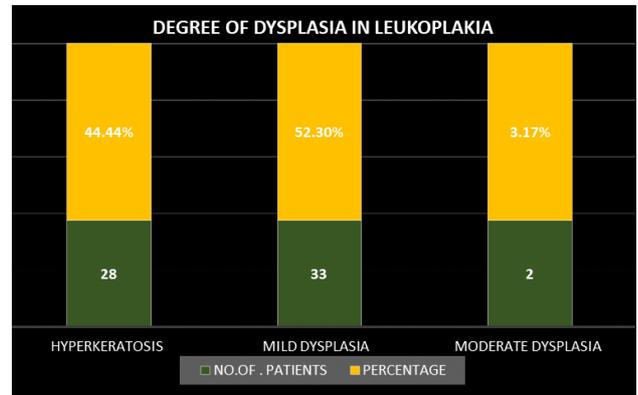


Fig 3: Degree of dysplasia in leukoplakia

Table 4: Estimation of Salivary Acetaldehyde in Smokers with Leukoplakia and Smokers without Leukoplakia

	Smokers with leukoplakia group A	Smokers without leukoplakia group B
Number of subjects	63	63
Mean salivary acetaldehyde levels (µl)	154.57	116.03
Standard deviation	9.592	4.789

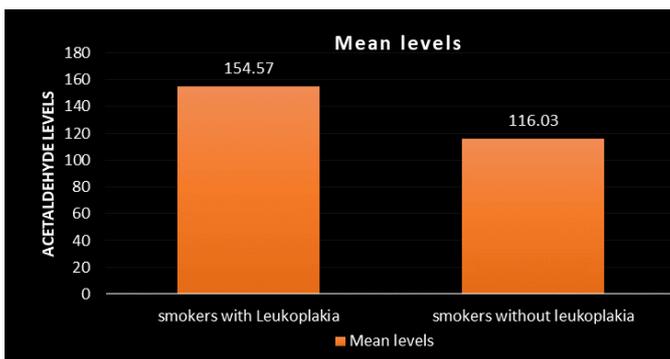


Fig 4: Mean levels

Table 5: Comparison of salivary acetaldehyde levels between smokers with leukoplakia and smokers without leukoplakia

	Smokers with leukoplakia	Smokers without leukoplakia
Number of subjects	63	63
Mean salivary acetaldehyde levels (µl)	154.57	116.03
Standard deviation	9.592	4.789
Mean difference (95% ci)	38.54 (35.86 – 41.21)	
T value	28.53	
P-value	<0.001	

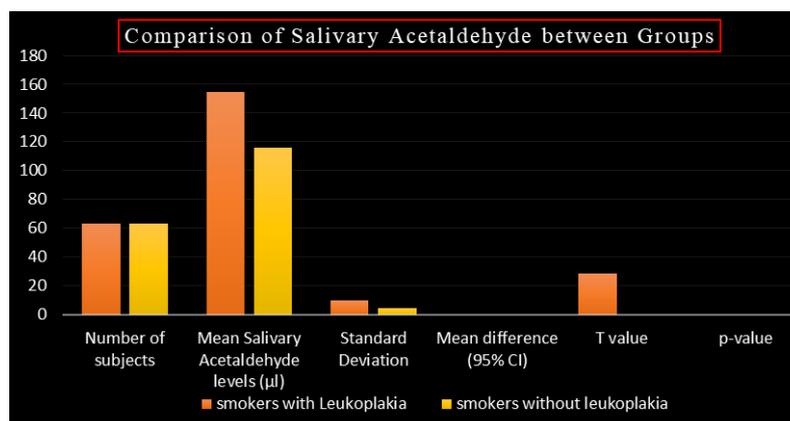


Fig 5: Comparison of salivary acetaldehyde between groups

Table 6: Correlation between histopathological grading and salivary acetalehyde

	Hyperkeratosis	Mild	Moderate
Number of subjects	28	33	2
Mean salivary acetaldehyde levels (µl)	157.83	153.26	154.0
Standard deviation	6.653	10.684	1.414
F value	1.43		
P-value	0.246		

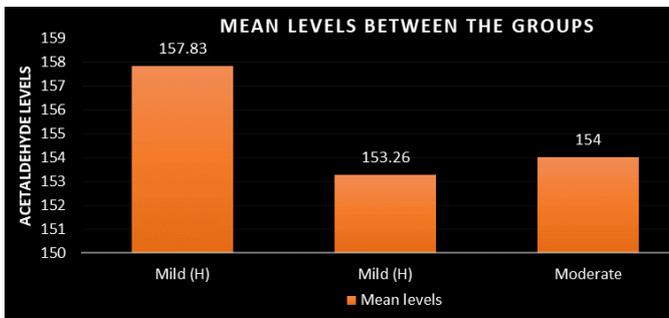


Fig 6: Mean levels between the groups

Discussion

Identification of lacunae related to salivary acetaldehyde in leukoplakia

Acetaldehyde is a well known carcinogen and is mainly studied in association with alcohol consumption. It is proved in many studies that alcohol consumption increases the level of acetaldehyde. On literature search there is sparse evidence found to identify the relationship of salivary acetaldehyde Level with smokers. Smoking is the most predominant etiology of leukoplakia and the tobacco smoke dissolves in saliva. Saliva has become unavoidable biomarker in the field of diagnosis. In order to fill the void we combined the factors smoking, leukoplakia and salivary acetaldehyde in our study to assess the amount of acetaldehyde (Carcinogen) produced and its correlation with leukoplakia.

Background evidence on acetaldehyde and carcinogen

The tests carried out by the International Agency for Research on Cancer (IARC) 2008 revealed carcinogenic property of Acetaldehyde on animals and acetaldehyde was classified. Into Group 2B (Possibly carcinogenic to humans) [23]. IARC in the following year (October 2009) declared that acetaldehyde present in alcoholic beverages thereof in vivo is classified as Group I carcinogen.

There is an increasing evidence that majority of tumor promotion action of alcohol is mediated by its first active toxic and carcinogenic metabolite acetaldehyde. Acetaldehyde is produced from ethanol in the epithelia by mucosal alcohol dehydrogenase yet higher levels are derived from the oxidation of microbial flora. Increase in salivary acetaldehyde level due to ethanol among smokers and alcoholics prove to be a reason for the synergistic carcinogenic action of alcohol [24].

Malignant tumors associated with acetaldehyde develop in those anatomic sites where acetaldehyde exposure is the most intense in the upper gastrointestinal tract, upper respiratory tract, esophagus and stomach [25]. Cancer development was seen in the nasal mucosa in a rat inhalation test at 3,000 ppm 6 hours/day, 5 days/week and 27 weeks. In the larynx of a hamster inhalation test for 7 hours/day, 5 days/week, 52 weeks acetaldehyde produced development of cancer. These test proves the carcinogenicity of acetaldehyde.

Comparison of study outcome with existing literature Age distribution

Age plays an important role in the clinical diagnosis and management of the disease. In the present study, 63 cases of oral leukoplakia were included as per the defined inclusion Criteria. The age distribution ranged from 20-70 years, with a mean age of 50.14 ± 10.76 years. Peak occurrence of Leukoplakia was observed between the age group of 51-60 years which is consistent with the study done by Banoczy (1977) and Wei Liu (2010).

Brad (2005) stated that less than 1% of men below the age of 30 have leukoplakia, but the Prevalence increases to an alarming 8% in men over the age of 70 [26].

Seamus *et al* (2008) suggested that the causes of leukoplakia are closely related to the Use of tobacco. The highest prevalence was found in the older age groups, 14.6% for those Aged 60 and above [27].

Estimation of salivary acetaldehyde levels

The salivary acetaldehyde levels were analyzed for 126 subjects using Gas chromatography technique. In our study we found the salivary acetaldehyde levels were considerably higher in smokers with leukoplakia which is on par to the study conducted by Salaspuro *et al* (2004). Author has suggested that increase alcohol consumption and smoking considerably increases the level of salivary acetaldehyde, thereby increasing the risk of upper gastro intestinal tract cancer. We in our study considered the potentially malignant disorder (leukoplakia) a definitive risk factor for cancer, similar to that of Salaspuro *et al* [28].

In the present study we found smoking an alternative etiological factor apart from alcohol which proves an increase in the salivary acetaldehyde level in contrast to the studies conducted by Lachenmeier *et al* (2009). The study of Lachenmeier stated that salivary acetaldehyde level increases on consumption of alcohol containing mouthwash excluding the habit of smoking. In our study we have established the fact that salivary acetaldehyde level is increased in patients who are smokers even without consumption of any alcohol containing mouthwash. This indicates strongly that smoking alone could be a definitive cause for increase of the carcinogen (acetaldehyde).

Seitz *et al* (2010) found out that the oral microbial flora (bacteria) oxidizes the ethanol to produce acetaldehyde. Salivary acetaldehyde level of concentration between 50 to 150 μl can be detected in saliva after ingestion of 0.5g/kg body weight of ethanol while it reduces considerably after using antiseptic mouthwash. In our study we measured the salivary acetaldehyde level with a mean value of 156 μl only from smokers without ingestion of ethanol. This suggest that smoking is a definite cause for increase in the salivary acetaldehyde level. The use of antiseptic mouthwash can be tried out in smokers to assess the level of variation in salivary acetaldehyde [29].

Salaspuro M *et al* (2011) suggested the concentration of salivary acetaldehyde level in smoker's increases over 1000 fold on comparison to other carcinogens. The acetaldehyde of tobacco smoke dissolves readily in the saliva during active smoking resulting in a mean of 260 μl acetaldehyde concentration in saliva. The cut off for mutagenic levels of acetaldehyde was 100 μl (4.4 mg/l), and this is exceeded over two-fold by smoking. In our study the acetaldehyde level in saliva had a highest mean value of 156 μl exceeding the mutagenic levels. The reason behind this difference in the mean value of acetaldehyde levels is possibly due to collection of salivary samples not during the active phase of smoking [30].

Salivary acetaldehyde was assessed using gas chromatography suggested by multiple studies. Values of salivary acetaldehyde in our study was estimated using the highly sensitive and specific Gas chromatography technique. Lesley *et al* (2011) found that gas chromatography has a sensitivity of $100\% \pm 11\%$ and a specificity of $95\% \pm 2.4\%$.

Kocealli *et al* (2014) proved a decrease in the salivary acetaldehyde levels due to improvement in oral hygiene. It

favors our study which suggest there is an increase in the salivary acetaldehyde level due to the habit of smoking. Smoking an important risk factor for periodontal diseases^[9]. Smoking thus compromises the oral hygiene causing an increase in the salivary acetaldehyde level. More over the study of Kocealli *et al* found that the salivary acetaldehyde level was higher in patients with oral cancer. In our study we found the salivary acetaldehyde levels were significantly higher in patients with leukoplakia. (Potentially malignant disorder) The results of our study and kocealli *et al* concludes that the salivary acetaldehyde levels will be higher in both potentially malignant disorder (leukoplakia) and oral cancer^[31].

Estimation of salivary acetaldehyde levels

The salivary acetaldehyde levels were analyzed for 126 subjects using Gas chromatography technique. In our study we found the salivary acetaldehyde levels were considerably higher in smokers with leukoplakia which is on par to the study conducted by Salaspuro *et al* (2004). Author has suggested that increase alcohol consumption and smoking considerably increases the level of salivary acetaldehyde, thereby increasing the risk of upper gastro intestinal tract cancer. We in our study considered the potentially malignant disorder (Leukoplakia) a definitive risk factor for cancer, similar to that of Salaspuro *et al*^[28].

Salivary acetaldehyde level and histopathological gradings

The histopathological grading of 63 patients (Group A) in our study was obtained from the department of Oral pathology and microbiology in an attempt to correlate with the level of salivary acetaldehyde. The obtained histopathological grading were hyperkeratosis, mild dysplasia and moderate dysplasia distributed in 28, 33 and 2 patients respectively. The salivary acetaldehyde values of the 28 cases of hyperkeratosis was $157.8 \pm 6.6 \mu\text{l}$, mild dysplasia was $153.3 \pm 10.6 \mu\text{l}$ while that of moderate dysplasia was $154 \pm 1.4 \mu\text{l}$. On comparing the mean values between the three groups it was found that the mean value of salivary acetaldehyde in hyperkeratotic patients was high than the mild dysplasia group. Though we obtained a slightly higher value of salivary acetaldehyde in hyperkeratosis group of patients than patients with mild dysplasia it shows no statistical significance. The comparison between the three histopathological grading was not statistically significant. ($P > 0.2$).

Our study is the first of its kind to correlate the histopathological grading between a carcinogen (Acetaldehyde) and a potentially malignant disorder (Leukoplakia)

Conclusion

The primary goal of the study was to estimate the most unnoticed carcinogen (Acetaldehyde) in saliva using the most accurate method of gas chromatography. The study arrived at a conclusion that smoking is a predominant etiology in the increase of salivary acetaldehyde levels. The unique feature of the study is it's the first of its kind in comparing the salivary acetaldehyde (Carcinogen) to a potentially malignant disorder (Leukoplakia). We obtained positive results in estimating the acetaldehyde levels among smokers with leukoplakia and smokers without leukoplakia. We also attempted in comparing the histopathological grading's of the potential malignant disorder (Leukoplakia) with the salivary acetaldehyde levels while we inferred results which were not significant statistically.

Thus in future studies might be carried out in concentrating on the level of carcinogen and its impact in the epithelial tissues of oral cavity.

References

1. Alaspuro VS, Alaspuro MS. Synergistic Effect Of Alcohol Drinking and Smoking on in Vivo Acetaldehyde Concentration In Saliva. 2004; 483:480-3.
2. Quertemont E, Didone V, Ph D, Didone V. Role of acetaldehyde in mediating the pharmacological and behavioral effects of alcohol. Alcohol Res Health. 2006; 29(4):258-65.
3. Sohnius E, Keck-wilhelm A, Lachenmeier DW. SHORT REPORT Salivary acetaldehyde increase due to alcohol-containing mouthwash use : A risk factor for oral cancer. 2009; (5):735-730.
4. Lachenmeier DW, Uebelacker M, Hensel K, Rehm J. Acetaldehyde in the human diet: An underestimated risk factor for cancer. Deut Leb Rundsch, 2010, 106.
5. Uebelacker M, Lachenmeier DW. Quantitative determination of acetaldehyde in foods using automated digestion with simulated gastric fluid followed by headspace gas chromatography. J Autom Methods Manag Chem [Internet]. 2011 Jan [cited 2015 Dec 12]; 2011:907317. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3124883&tool=pmcentrez&rendertype=abstract>
6. Guan X, Rubin E, Anni H. NIH Public Access. 2013; 36(3):398-405.
7. Ramdzan AN, Mornane PJ, McCullough MJ, Mazurek W, Kolev SD. Determination of acetaldehyde in saliva by gas-diffusion flow injection analysis. Anal Chim Acta [Internet]. Elsevier B.V. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/23790294>. 2013; 786:70-7.
8. Kocaelli H, Apaydin A, Aydil B, Ayhan M, Karadeniz A, Ozel S, *et al*. Evaluation of potential salivary acetaldehyde production from ethanol in oral cancer patients and healthy subjects. Hippokratia. 2014; 18:269-74.
9. Auluck A, Pai KM. (Mis) nterpretations of leukoplakia. J Can Dent Assoc [Internet] Available from: <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med5&NEWS=N&AN=15871769>. 2005; 71(4):237-8.
10. van der Waal I. Oral leukoplakia, the ongoing discussion on definition and terminology. Med Oral Patol Oral Cir Bucal. 2015; 20(6):685-92.
11. Parlatescu I, Gheorghe C, Coculescu E, Tovar S. Oral leukoplakia-an update. Mædica [Internet]. 2014; 9(1):88-93. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4268300&tool=pmcentrez&rendertype=abstract>
12. Dysplasia K. Leuloplakia-Review of a Potentially Malignant Disorder. 2014; 8(8):4-7.
13. Amagasa T, Yamashiro M, Ishikawa H. Oral Leukoplakia Related to Malignant Transformation. Oral Sci Int [Internet]. Japanese Stomatological Society. 2006; 3(2):45-55. Available from: [http://dx.doi.org/10.1016/S1348-8643\(06\)80001-7](http://dx.doi.org/10.1016/S1348-8643(06)80001-7)
14. Van der Waal I. Potentially malignant disorders of the oral and oropharyngeal mucosa; terminology, classification and present concepts of management. Oral Oncol. 2009; 45(4-5):317-23.
15. Feller L, Lemmer J. Oral leukoplakia as it relates to HPV infection: A review. Int J Dent. 2012; 2012.

16. Mohammed F, Fairozekhan AT. Leukoplakia, Oral, 2017.
17. Van der Waal I, Schepman KP, Van der Meij EH. A modified classification and staging system for oral leukoplakia. Oral Oncol. 2000; 36(3):264-6.
18. Sadiq H, Gupta P, Singh N, Thakar SS, Prabhakaran I, Thakral J. *et al* Various Grading Systems of the Oral Epithelial Dysplasia : A Review. 2015; 1(11):20-6.
19. Stackler B, Christensen EN. Quantitative determination of ethanol. 1974; 25(4).
20. Moore L, Wicks J, Spiehler V, Holgate R. Gas Chromatography-Mass Spectrometry Confirmation of Cozart RapiScan Saliva Methadone and Opiates Tests. 2011; 25:520-4.
21. Tiscione NB, Alford I, Yeatman DT, Shan X. Ethanol analysis by headspace gas chromatography with simultaneous flame-ionization and mass spectrometry detection. J Anal Toxicol. 2011; 35(7):501-11.
22. Charapitsa SV, Zadreyko YV, Sytova VHSN. estimation of the accuracy of method for quantitative determination of volatile compounds in alcohol products, 2016.