10.5005/jp-journals-10024-1221

ORIGINAL RESEARCH

Effect of Methylxanthines (Coffee/tea Consumers) on Oral Precancer and Oral Cancer Patients with Smoking and Smokless Tobacco Habits

Yasmeen F Zubairy, Vinayak W Patil, Tabita Benjamin, Daya Jangam, Mohammed Nadeem Ahmed Bijle Shankargouda Patil

ABSTRACT

Aim: To study, whether the consumption of regular tea/coffee (methylxanthines) increases the risk of oral cancer in patients with smoking and smokeless tobacco habits.

Materials and methods: This study was conducted on a total of 90 oral cancer and precancerous patients, from western Maharashtra (India) males in the age group of 20 to 45 years who were with smoking and smokeless tobacco habits; also regular tea/coffee consumers were subjected to biochemical parameters such as aspartate transaminase (AST) and alanine transaminase (ALT) from saliva and serum of patients with oral precancer (submucous fibrosis, leukoplakia) and oral cancer patients and compared with 90-age and sex-matched controls. Individuals consent was taken to measure their biochemical parameters, by using Hafkenscheid method in whole saliva and serum. Statistical analysis of variance (ANOVA) with Tukey's correction for multiple group comparisons was performed using Student t-test.

Results: Results show, that a statistically significant increase in value ($p < 0.05$) in ALT, AST in both saliva and serum was observed in precancerous and oral cancer patients among the study group as compared to the control group.

Conclusion: In the present study, there was increase in the levels of ALT, AST enzymes in both saliva and serum levels in the study group as compared to the control group which was statistically significant ($p < 0.05$) suggesting that long-term exposure of methylxanthines results in impairment of salivary gland antioxidant system which may affect the anticarcinogenic action of saliva.

Clinical significance: Oral fluids may be utilized effectively to study the variations in the biochemical constituents of saliva of leukoplakia, submucous fibrosis and oral cancer patients.

Keywords: Methylxanthines, Alanine aminotransferases, Aspartate aminotransferases, Oral cancer.

How to cite this article: Zubairy YF, Patil VW, Benjamin T, Jangam D, Bijle MNA, Patil S. Effect of Methylxanthines (Coffee/tea Consumers) on Oral Precancer and Oral Cancer Patients with Smoking and Smokless Tobacco Habits. J Contemp Dent Pract 2012;13(6):745-748.

Source of support: Nil

Conflict of interest: None declared

INTRODUCTION

Humans are continuously exposed to various foreign chemicals, such as drugs, food additives and pollutants. These substances interfere with digestion, absorption and metabolism by producing toxic effects in tissues like liver as well as in saliva.[1,2] Methylxanthines (caffeine, theophylline and theobromine) are being consumed in the form of tea, coffee, coco and beverages which has become a major issue in the 20th century. Use of tobacco has become a major risk factor for the development of oral cancer, with a worldwide incidence of over 300,000 new cases annually.[3]

Tobacco was first introduced to Western civilization by the Spanish explorers of America in the early sixteenth century. Toward the beginning, it was usually smoked in pipes, but later on as it became more common people started to chew and even snuffed.[4] It has been established that if it continues further, more people will die annually from tobacco-related illness than from any single disease.[5]

Tobacco contains potent carcinogens, including nirtosamines (nicotine), polycyclic aromatic hydrocarbons, nitrosoproline, polonium, carbon monoxide, thiocyanate, hydrogen cyanide, nicotine and metabolites of these constituents.[6] These procarcinogens are DNA toxic and result in the promotion of oral cancer.[7] The 'field cancerization' concept is the currently accepted explanation for the carcinogenic effect of cigarette smoke on oral mucosa.[8] According to this theory, there is a constant and direct attack of various cigarette smoke reagents on the oral epithelial cells, which gradually accumulate and cause a stepwise malignant transformation. It has been suggested that free radicals, reactive oxygen species and reactive

nitrogen species in the inhaled cigarette smoke induce this gradually evolving process, initially expressed by dysplastic lesions of the mucosa, are then transformed into carcinoma *in situ* lesions and eventually result in full-blown infiltrating and metastasizing oral carcinoma. Based on these facts the study was undertaken to compare and analyze the role of transaminases enzymes (ALT, AST) in saliva and serum of the precancerous and oral cancer patients.

MATERIALS AND METHODS

With the patient's permission via written consent we conducted a study of 90 males from 20 to 45 years of age. The patients were divided into three categories; submucous fibrosis, leukoplakia and malignant oral cancer through histopathological examination. Our research was executed by comparing the group of diagnosed patients with a control group of 90 healthy males with an identical age group.

The control group consisted of healthy males who displayed normal dietary habits and consumed tea and coffee. The patients were males who consumed tobacco in different forms (smoke and smokeless) who were diagnosed with precancer as well as oral cancer.

Control group participants who had any history of illness, addiction to toxic substances as well as prescribed medication were excluded from the study. The study group subjects were of average socioeconomic status (middle class). The demographic, personal, clinical history, the dietary intake was made with the help of history card prepared as soon as the subjects were scrutinized. Saliva was collected from the subjects as well as random 10 ml venous blood in EDTA bulb. Serum was analyzed using standard method of Hafkenscheid.[9,10]

Estimation of salivary AST (SGOT) and ALT (SGPT) was analyzed with standard method of Hafkenscheid.[9,10] The AST (SGOT) and ALT (SGPT) was measured by using reagents of M/S Accurex Biomedical Ltd. In order to measure the amounts of enzymes in each sample 10 ml of saliva was collected and centrifuged at 5,000 rpm to remove excess mucus and unwanted particles. After the centrifugation process the supernatants were harvested in order to determine the amounts of AST (SGOT) and ALT (AGPT) enzymes. In order to complete the measurements the M/S Accurex Biomedical Ltd enzyme reagent was diluted and the saliva samples were added to the solution. The solution was then mixed gently to dissolve the enzymes and the date of preparation was recorded. This is necessary because the working solution is stable for 3 days at 2 to 8°C. The required amount of solution was warmed to 37°C before use.

Serum/plasma 0.1 ml and working solution 1.0 ml were mixed thoroughly and the assay mixture was transferred immediately to the thermostated cuvette with starting the stopwatch simultaneously. First reading was recorded at 60th second. Subsequently three more reading were recorded with 30-second interval at 340 nm.

RESULTS

A comparison of the salivary enzyme activities shows increased activity of ALT, AST by 2, 12, 22 and 9, 18, 34% respectively as observed in all three cases (Table 1) in saliva and serum compared to control patients which are statistically significant ($p < 0.05$) in the leukoplakia, submucous fibrosis and oral cancer patients when compared with that of the control groups. Similarly when the percentage difference was calculated (Table 2) a statistically

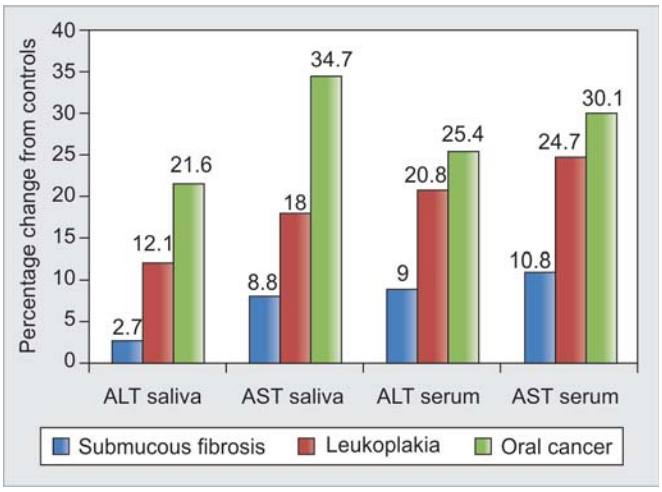
Table 1: Mean values of different groups using saliva and serum

Parameters	*Control group (N = 90)*	*Precancerous group*		*Oral cancer (group)*
		Submucous fibrosis (N = 30)	*Leukoplakia (N = 30)*	*(N = 30)*
ALT saliva (U/L)	23.50	24.14	26.35	28.58
AST saliva	21.45	23.34	25.32	28.89
AST serum (U/L)	16.56	18.35	20.65	21.55
ALT (U/L) serum	17.66	19.25	21.34	22.15

Table 2: Mean values of different groups using saliva and serum

Parameters	*Percentage change with reference to controls*		
	Submucous fibrosis group (%)	*Leukoplakia group (%)*	*Oral cancer group (%)*
ALT saliva (U/L)	2.7	12.1	21.6*
AST saliva	8.8	18.0*	34.7*
AST serum (U/L)	10.8	24.7*	30.1*
ALT (U/L) serum	9.0	20.8*	25.4*

Alanine transaminase (ALT), aspartate transaminase (AST); Values represent the mean percentage change with respect to the control group; *$p < 0.05$ (student's t-test) indicates statistical significance of difference from the control group



Graph 1: The mean values of percentage difference from control group for different groups using saliva and serum ($p < 0.05$ (student's t-test) indicates statistical significance of difference in different groups)

significant increased progress graph in the form of histograms was plotted (Graph 1) to establish the percentage change by comparing the variations in the submucous fibrosis, leukoplakia and oral cancer groups as compared to the control groups.

DISCUSSION

Methylxanthines (theophylline, theobromine,caffeine) consumption in the form of tea, coffee, coco and cola beverages have recently been studied by several research workers.[11,12] Methylxanthines are consumed by large population in high amounts followed by tobacco, both smoked as well as smokless. Tobacco use has become a major risk factor in case of oral squamous carcinoma with worldwide cases of 300,000 every year.[6] Methylxanthines (including theophylline, theobromine, caffeine and nicotine) are plant-derived alkaloids with ubiquitous use in beverages (caffeine in coffee and soda), foods (theobromine in chocolate), tobacco products (nicotine) and medications (theophylline and caffeine). Oral carcinoma are related to certain alkaloids that give rise to nitrosamines which are shown to be carcinogenic.[13]

All the subjects in this study were habitual tobacco users both smoking tobacco as well as smokeless tobacco users, the salivary enzymes levels of ALT and AST were studied which showed a statistically significant increase in the enzymes. This can be correlated to a study which stated that there was an increased levels of ALT/AST in the smokers. [3] Saliva has the capacity of detoxifying the carcinogen by inducing phase 2 xenobiotic enzymes, which becomes an important mechanism by which cancer risk can be attenuated. It is also stated that during glucuronidation reactions the transferases constitute a detoxification process by aiding the excretion of complexes which becomes a risk factor in the development of oral cancer.[14] However, an additional mechanism could be a reduction in the activation of environmental procarcinogens by certain cytochrome P450 (CYP) isoforms.[15] A comparison of the salivary enzyme activities shows increased activity of ALT by 2, 12 and 22% which was observed in all three cases (Table 2) in saliva. Similarly it was as compared to control patients. Similarly, AST from saliva showed a significant increase by 9, 18 and 34% as compared to the control group (Table 2). Serum AST and ALT were also increased in the study group as compared to control group. This suggests impairment of the salivary gland function affecting the anticarcinogenic activity of the saliva. In case of the transaminases enzymes activities in serum may be increased due to the damage of the liver by various metabolites.

CONCLUSION

From the above study it can be concluded that that methylxanthines can have an effect on salivary enzymes ALT, AST. This may increase the risk of malignant transformation along with the addition of the tobacco metabolites, and hence the anticarcinogenic effect of saliva is impaired. Serum ALT, AST increased levels result due to the damaged liver function.

If the patients have both the habits of tea and tobacco than salivary enzymes levels can also be used as biomarkers. All these results indicate that when individuals who consume tobacco along with tea/coffee are at a higher risk to the development of cancer show toxic response. Salivary ALT and AST can be used as markers in diagnosis.

CLINICAL SIGNIFICANCE

All these results indicate that individuals who consume tobacco along with tea/coffee are at a higher risk to the development of cancer and show toxic response. Salivary ALT and AST can be used as markers in diagnostic tools in establishing a relationship and to study the biochemical variations in case of submucous fibrosis, leukoplakia and oral cancer patients. Saliva has been discussed lately as an important biological material which can be utilized in introducing new diagnostic tests that may contribute to diagnostic tools.

REFERENCES

1. Bachmann K, Sanyal G, Potter J, Schiavone R, Loch J. In vivo evidence that theophylline is metabolized principally by CYP1A in rats. Pharmacology 1993 Jul;47(1):1-7.
2. Robson RA, Miners JO, Matthews AP, Stupans I, Meller D, McManus ME, et al. Characterisation of theophylline metabolism by human liver microsomes. Inhibition and immunochemical studies. Biochem Pharmacol 1988 May 1;37(9):1651-59.

3. Sankarnarayan R, Masuyaer E, Swaminathan R, Ferley. Head and neck cancer a global perspective on epidemiology and prognosis. Anticancer Res 1998;18:4779-86.
4. Silverm S. Oral cancer (4th ed). Hamilton; BC Becker 1998; 25-40.
5. Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Recommended methods for the determination of four enzymes in blood. Scand J Chin Lab Invest 1974;33:291-306.
6. Choo RE, Huestis MA. Oral fluid as a diagnostic tool. Clin Chem Lab Med 2004;42(11):1273-87.
7. Dayan D, Hirshberg A, Kaplan I, Rotem N, Bodner L. Experimental tongue cancer in desalivated rats. Oral Oncol 1997 Mar;33(2):105-09.
8. Nishioka H, Nishi K, Kyokane K. Human saliva inactivates mutagenicity of carcinogens. Mutat Res 1981 Oct;85(5):323-33.
9. Hafkenscheid JC, Vander Ven-Jongerkrjing J. Influence of pyridoxa-5-phosphate on the determination of alanine aminotransferas and aspartate aminotransferase of commercial test sera. J Clin Chem Clin Biochem 1979 Apr;17(4):219-23.
10. Zhang L, Xiao H, Wong DT. Salivary biomarkers for clinical applications. Mol Diagn Ther 2009;13(4):245-59.
11. Fuhr U, Doehmer J, Battula N, Wolfel C, Kudla C, Keita Y, et al. Biotransformation of caffeine and theophylline in mammalian cell lines genetically engineered for expression of single cytochrome P450 isoforms. Biochem Pharmacol 1992 Jan 22;43(2):225-35.
12. Govidwar SP, Kachole MS, Pawar SS. Effect of caffeine on the hepatic microsomal mixed function oxidase system during Phenobarbital and benzo [a] pyrene treatment in rats. Toxicol Lett 1988 Aug;42(2):109-15.
13. Lippman SM, Hong WK. Molecular markers of risk of oral cancer. N Engl J Med 2001 Apr 26;344(17):1323-26.
14. Guengerich FP. Human cytochrome P450 enzymes. In Cytochrome P450. In: Ortiz de Montellano (Ed). Plenum Publishers, New York 1995:473-535.
15. Mirbod SM, Ahing SI. Tobacco associated lesions of oral cavity part 1: Nonmalignant lesions. J Can Dent Assoc 2000 May;66(5):252-56.

ABOUT THE AUTHORS

Yasmeen F Zubairy

PhD Student, Department of Biochemistry, Grant Medical College and Sir JJ Groups of Hospital, Mumbai, Maharashtra, India

Vinayak W Patil

Professor and Head, Department of Biochemistry, Grant Medical College and Sir JJ Groups of Hospital, Mumbai, Maharashtra, India

Tabita Benjamin

Professor, Department of Oral Pathology, Government Dental College Mumbai, Maharashtra, India

Daya Jangam

Professor and Head, Department of Oral Medicine and Radiology MA Rangoonwala College of Dental Sciences and Research Centre Pune, Maharashtra, India

Mohammed Nadeem Ahmed Bijle

(Corresponding Author)

Postgraduate Student, Department of Pedodontics and Preventive Dentistry, MA Rangoonwala College of Dental Sciences and Research Centre, Pune, Maharashtra, India, Phone: 9822884874, e-mail: info@drnadeembijle.com/nbijle@yahoo.co.in

Shankargouda Patil

Assistant Professor, Department of Oral Pathology, MS Ramaiah Dental College and Hospital, Bengaluru, Karnataka, India