DOI: 10.7860/JCDR/2015/13928.6763

Original Article

Dentistry Section

Investigation of the Possibility of Using Serum Ischemia Modified Albumin (IMA) as a Novel and Early Marker of the Extent of Oxidative Stress Induced by Various Tobacco Products

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ABSTRACT

Background: Ischemia Modified Albumin (IMA) is an altered serum albumin that forms under the conditions of oxidative stress and is considered as a biomarker of cardiac ischemia. The objective of this study was to evaluate the ischemia modified albumin (IMA) in the serum of the individuals with different types of tobacco habits in order to investigate the possibility of using this as a biomarker for the oxidative stress induced by the tobacco products.

Materials and Methods: The study included 90 subjects, who were Grouped as control (30), Group I (betel quid chewers), Group II (gutkha chewers), Group III (smokers) and Group IV (mixed). Serum was collected from subjects of all Groups and IMA estimation was done using Albumin Cobalt binding assay. The results were tabulated and analysed statistically.

Results: The mean serum IMA levels in control, Group I, Group II, Group III and Group IV were 0.52547 ABSU, 0.68767 ABSU, 0.47433 ABSU,0.36540 ABSU and 0.54593 ABSU respectively.

Conclusion: The results show that serum IMA levels were increased in betel quid chewers and mixed Group compared to the controls. From the results noted in this study we suggest that IMA can be used as an early marker for tobacco related oxidative stress.

Keywords: Antioxidants, Carcinogenesis, Reactive oxygen Species (ROS), Tobacco specific nitrosamines (TSN's)

INTRODUCTION

The smoking and chewing of tobacco alone or with betel leaf, has a significant detrimental impact on oral tissues. Tobacco contains chemical constituents which are known carcinogens. The habit of tobacco consumption has been clearly associated with potentially malignant diseases of oral cavity and World Health Organization (WHO) has estimated that 90% of oral cancers in India among men are attributable to tobacco habits [1].

Both smoke and smokeless form contains large amount of tobacco specific nitrosamines (TSN's) which are known carcinogens that can have direct mutagenic effect on human oral mucosal cells. Investigations in cell culture studies have demonstrated that besides carcinogenic TSN's, reactive oxygen species (ROS) derived from tobacco and oxidative DNA damage are also critical for the pathologies of tobacco induced cancers. These radicals play an important role in the development of cancer by causing the DNA damage, by activating the procarcinogens, by altering the cellular antioxidant defence system and also they can play the role of initiators and/or promoters of carcinogenesis [2].

Ischemia Modified Albumin (IMA) is a novel protein, that has been extensively studied in ischemic heart diseases and the measurement has been approved by the Food and Drug Authority (FDA) for extent of cardiac ischemia. Although many lipid peroxidation products (LPOs) and antioxidant enzyme activities have been studied to assess the extent of oxidative stress induced by tobacco, so far no attempt has been made to estimate IMA as a marker for tobacco induced oxidative stress. With this background, the importance of a study in this direction was recognized and the present study was designed to estimate the albumin and IMA in serum of individuals with different tobacco related habits to compare and establish a relationship between the levels of albumin, IMA, and their ratio in serum in order to explore the possibility of one or more of the above mentioned parameters being an early marker for tobacco related oxidative stress.

OBJECTIVES

The objectives of the study were the estimation of serum albumin levels in subjects who use different forms of tobacco, to estimate serum Ischemia Modified Albumin (IMA) levels in the same subjects, to calculate the IMA/Albumin ratio in serum of these subject and to compare and establish a relationship between the levels of Albumin, IMA, and their ratio in serum in order to explore the possibility of one or more of the above mentioned parameters being an early marker for tobacco related oxidative stress.

MATERIALS AND METHODS

The study was conducted in the Department of Oral Pathology and Microbiology, Yenepoya Dental College, Deralakatte, Mangalore in the year 2012.

Sixty healthy individuals with various tobacco related deleterious oral habits were included in the study, chosen from the local residents by random sampling method. Age and sex matched, 30 healthy volunteers without tobacco habits were selected for the control Group. The individuals with systemic diseases, abnormal endocrinal & immunological status and those exposed to chemicals as a result of occupation were excluded from the study.

ETHICAL APPROVAL

Ethical approval to carry out this study was obtained from the Yenepoya University Ethics committee. The purpose of the study, and the procedures to be carried out, were explained to the study subjects and only those who agreed to give a written consent were included in the study.

Methods of Collection of Data

The selection of the subjects for the study was done, based on the history of habits. After obtaining detailed history of their habits, a total of 60 individuals who were using tobacco in different forms from more than 5 years were selected as study Group. Selected individuals were categorized into the following Groups –

Study Group

Group I (Betel quid chewers): Those with traditional betel quid chewing habit (tobacco along with betel leaf and slaked lime). (n =15).

Group II (Gutkha chewers): Those using commercial tobacco products (gutkha sachets) (n =15).

Group III (Smokers): Those with habit of smoking tobacco (beedi/ cigarette). (n =15).

Group IV (Mixed): Those with combination of tobacco habits. (n =15).

Control Group: Individuals without any tobacco related habits. (n=30).

After recording detailed habit history, oral examination of the study subjects was carried out using diagnostic instruments (using mouth mirror & probe). If tobacco related mucosal lesions are present, it was noted. After explaining the details of the study, blood samples were collected for analysis.

A 5ml of peripheral blood sample were collected from each patient with disposable syringes under aseptic conditions through venipuncture. Serum was separated from the blood by centrifugation at 3000 rpm. The samples thus collected were stored below zero degrees until analysed for albumin and IMA.

To estimate serum albumin levels Bromocresol Green (BCG) kit was used [3]. IMA was estimated in serum using Albumin Cobalt Binding Assay [4]. It is a manual colorimetric assay to assess the ability of binding exogenous cobalt Co (II) to human albumin in serum. A 50 µL water solution of 0.1% cobalt chloride ($CoCl_2.6H_2O$) was added to 200 µL of serum, gently mixed and kept in dark for 10 minutes (for adequate cobalt albumin binding), then 50 µL of dithiothreitol (DTT) solution (1.5 mg/mL H_2O) was added as a colorizing agent. The reaction was quenched two minutes later by adding 1.0 mL of 0.9% NaCl. The blank was prepared similarly with the exclusion of DTT. Specimen absorbencies were analysed at 470nm using a spectrophotometer. IMA was calculated from the difference between samples measured with and without DTT. The results were reported as absorbance units (ABSU) [4].

RESULTS

The mean serum IMA levels in control, Group I, Group II, Group III and Group IV were 0.52547 ABSU, 0.68767 ABSU,0.47433 ABSU,0.36540 ABSU and 0.54593 ABSU respectively [Table/ Fig-1]. Comparison of the result between the Groups using Tukey HSD Multiple Comparisons [Table/Fig-2] shows that the difference in IMA levels was found to be statistically significant (p≤0.05) between Group I and Group II) and highly significant (p≤ 0.001) between Group I and Group III. The mean serum Albumin levels in control, Group I, Group II, Group III and Group IV were 4.733 gm/ dL, 4.7467 gm/dL, 4.7467 gm/dL, 4.9067 gm/dL and 4.5733 gm/ dL respectively [Table/Fig-3]. The mean serum IMA/Albumin ratio in Group I, Group II, Group III, Group IV and Group V were 0.1123, 0.1460, 0.1007, 0.0744 and 0.1202 respectively [Table/Fig-4].

DISCUSSION

Co-relation between tobacco usage and occurrences of oral potentially malignant diseases and oral cancer is well-recognised. Heavy tobacco users have a significantly increased incidence of these lesions [5]. Tobacco products which were usually consumed by a small section of the population are today part of the modern urban and rural lifestyle and are consumed in various forms such as smoking (beedis/cigarettes/pipes), chewing (betel quid/gutkha), snuff, etc. Therefore in the present study subjects with these habits were included.

It is a well-established fact that use of tobacco in various forms is an important aetiologic factor in the development of oral cancer [6]. Possible mechanisms by which tobacco induces carcinogenesis are TSN's and the related genotoxicity and oxidative stress induced by various chemicals leached out during smoking and chewing [7]. Two popular chewing habits among Indian population are: (i) betel quid chewing, a mixture of areca nut (*Areca catechu*), catechu (*Acacia catechu*), tobacco and slaked lime {calcium oxide (CaO) and calcium hydroxide ($Ca(OH)_2$)} wrapped in a betel leaf (*Piper beetle*); and (ii) Gutkha which is available in sachets is a mixture of areca nut, catechu, slaked lime and tobacco with added sweeteners and flavouring agents. Studies have shown that the ROS, implicated in multistage carcinogenesis, are generated in ample quantities in the oral cavity during tobacco chewing [8]. Nair et al., first demonstrated that in the alkaline pH(>9.5), aqueous extracts of areca nut and catechu will produce superoxide anion and hydrogen peroxide [7]. The presence of $Ca(OH)_2$ in slaked lime leads to alkaline conditions in the oral cavity, favouring ROS generation.

Studies have shown that one puff of tobacco smoke contains approximately 10^{15} radicals (alkyl & peroxyl types and nitric oxide), thus exposing the oral environment to a state of oxidative stress [9]. Hence, it is well-understood that, the levels of oxidative stress created in the oral cavity by different tobacco related habits vary to a greater extent and may be detectable as a localized or systemic alteration in oxidative biomarker.

IMA is a protein that is proved as a marker of oxidative stress by many studies [10,11]. Serum albumin with a decreased cobalt

Groups	n	Mean±Std. Deviation	Minimum	Maximum	p value
Control	30	0.52547±0.142707	0.268	0.918	0.0005 (HS)
Betel quid	15	0.68767±0.221652	0.204	1.026	
Gutkha	15	0.47433±0.186398	0.115	0.790	
Smokers	15	0.36540±0.239998	0.030	0.857	
Mixed	15	0.54593±0.148103	0.313	0.814	
Total	90	0.52071±0.203915	0.030	1.026	

[Table/Fig-1]: Comparison of serum IMA levels in the study Groups. Statistical analysis: by One-way ANOVA; Significance: F=6.072; p=0.0005.

(I) Groups	(J) Groups	Mean Difference (I-J)	p	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Control	Betel quid	-0.162200	0.050	-0.32439	0.00000
	Gutkha	0.051133	0.904	-0.11106	0.21332
	Smokers	0.160067	0.055	-0.00212	0.32226
	Mixed	-0.020467	0.997	-0.18266	0.14172
Betel quid	Gutkha	0.213333	0.017 (S)	0.02605	0.40062
	Smokers	0.322267	0.0005 (HS)	0.13498	0.50955
	Mixed	0.141733	0.226	-0.04555	0.32902
Gutkha	Smokers	0.108933	0.488	-0.07835	0.29622
	Mixed	-0.071600	0.824	-0.25888	0.11568
Smokers	Mixed	-0.180533	0.064	-0.36782	0.00675

[Table/Fig-2]: Comparison of serum IMA levels between the study Groups. Statistical analysis: Tukey HSD Multiple Comparisons between the five Groups; Level of statistical significance: p≤0.05 was considered significant (S); p≤ 0.001 was considered highly significant (HS).

Groups	N	Mean± Std. Deviation	Minimum	Maximum	p value
Control	30	4.7333±0.51215	3.80	5.70	0.442 (NS)
Betel quid	15	4.7467±0.45177	4.10	5.60	
Gutkha	15	4.7467±0.29488	4.30	5.50	
Smokers	15	4.9067±0.56753	4.10	5.80	
Mixed	15	4.5733±0.43172	4.00	5.60	
Total	90	4.7400±0.46971	3.80	5.80	

[Table/Fig-3]: Comparison of serum albumin levels in the study Groups. Statistical analysis: by One-way ANOVA; Significance:F=0.945 p=0.442 (NS= not significant)

Groups	N	Mean±Std. Deviation	Minimum	Maximum	p-value
Controls	30	0.1123± 0.03421	0.05	0.18	0.0005 (HS)
Betel quid	15	0.1460± 0.04949	0.04	0.24	
Gutkha	15	0.1007± 0.04162	0.02	0.17	
Smokers	15	0.0744± 0.04919	0.01	0.17	
Mixed	15	0.1202± 0.03575	0.07	0.19	
Total	90	0.1110± 0.04567	0.01	0.24	

[Table/Fig-4]: Comparison of IMA / albumin ratio in serum in the study Groups. Statistical analysis: by One-way ANOVA; Significance: F=6.097; p=0.0005

binding capacity as a result of ischemic events is referred to as IMA. The generation of ROS, modifies the N-terminal region of albumin to yield increased levels of IMA [4]. It is conceivable that greater the magnitude of ROS greater is the levels of IMA. Even though several LPO products and antioxidant enzyme activities have been studied to determine the degree of oxidative stress induced by tobacco [12,13], so far no attempt has been made to estimate IMA as a marker for tobacco induced oxidative stress.

Therefore, the present study was conducted to assess the extent of the oxidative stress induced by different tobacco habits by estimating serum levels of this novel oxidative stress biomarker, IMA. An attempt is also made to compare and establish a relationship between the levels of Albumin, IMA, and their ratio in serum in order to explore the possibility of one or more of the above mentioned parameters being an early marker for tobacco related oxidative stress.

The results show that serum IMA levels were increased in betel quid chewers and mixed Group compared to the controls, while, lesser in other study Groups. It has been reported that elevated IMA levels will return to normal within a few hours, with the removal of free radicals or an accelerated clearance [14]. As the raise in IMA levels is transient and it has a short half life, the IMA level which would increase within few minutes after use of tobacco and related substances may come down after clearing the free radicals. Therefore, it is reasonable to consider, the variation in the time gap between the last use of tobacco and sample collection as a reason for inconsistency in the result obtained, which was a limitation in this study. To overcome this problem standardization of the time gap between the use of tobacco and collection of samples is required to find out the exact relationship between these parameters and oxidative stress induced by tobacco use.

Slight increase in serum IMA noted in betel quid chewers could be due to the high concentration of slaked lime used in betel quid which acts to keep the active ingredients in its freebase or alkaline form, enabling it to enter the bloodstream via sublingual absorption and therefore imparting a systemic effect [15].

From the results noted in this study we suggest that IMA can be used as an early marker for tobacco related oxidative stress. Although utmost care was taken in all steps of the study process including collection, transportation, storage and analysis, there is possibility of some errors. To the best of our knowledge there are no studies reported in the literature where a comparison of serum IMA levels and their relationship to the levels of albumin between the different tobacco user Groups is done. This is a preliminary report in which we attempted to investigate whether IMA can be used as a tobacco induced oxidative stress biomarker. Therefore there is necessity for further research in this direction using more sensitive techniques and more standardized conditions to confirm the results obtained in this study and substantiate that IMA can be early marker for tobacco related oxidative stress.

To confirm the prognostic significance of this marker, further studies are required including a larger sample size in each of study Groups, subjects with long term tobacco use, and patients with tobacco related potentially malignant oral diseases and oral cancer.

CONCLUSION

Serum IMA levels in different study subjects did not show much alteration from controls. However, slight alteration was noted in betel quid chewers, which may be related to high concentration of slaked lime causing increased alkalinity, favouring better sublingual absorption of chemical constituents. Since IMA levels were increased after exposure to tobacco which is known to cause oxidative stress, it can be considered as a reliable marker for tobacco induced oxidative stress. However, further research works are required to support our observations.

ACKNOWLEDGEMENTS

The authors acknowledge Indian Council of Medical Research (ICMR), New Delhi, India, for the financial support.

REFERENCES

[1] Peterson PE. Strengthening the prevention of oral cancer: the WHO perspective. *Community Dentistry Oral Epidemiology*. 2005;33:397-99.
[2] Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radic Biol Med*. 1990;8(6):583-99.
[3] Kumar A, Sivakanesan R, Singh S. Oxidative stress, endogenous antioxidant and ischemia modified albumin in normolipidemic acute myocardial infarction patients. *Journal of Health Science*. 2008;54(4):482-87.
[4] Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia- a preliminary report. *J Emerg Med*. 2000;19:311-15.
[5] Nair U, Bartsch H, Nair J. Alert for an epidemic of oral cancer due to use of the betel quid substitutes gutkha and pan masala: a review of agents and causative mechanisms. *Mutagenesis*. 2004;10(4):251-62.
[6] Tobacco Habits Other than Smoking; Betel quid and Areca-nut Chewing; and Some Related Nitrosamine. IARC Monographs on the Evaluation of Carcinogenic Risks of Chemicals to Humans.1985;37.
[7] Nair UJ, Nair J, Mathew B, Bartsch H. Glutathione S-transferase M1 and T1 null genotypes as risk factors for oral leukoplakia in ethnic Indian betel quid/tobacco chewers. *Carcinogenesis*.1999;20:743-48.
[8] Mehrotra R, Yadav S. Oral squamous cell carcinoma: Aetiology, pathogenesis and prognostic value of genomic alterations. *Indian Journal of Cancer.* 2006;43(2):60-66.
[9] Obradovic LN, Pešut D, Trifunovic VS, Adžic T. Influence of tobacco smoke on the appearance of oxidative stress in patients with lung cancer and chronic obstructive pulmonary diseases. *Vojnosanit Pregl*. 2006;63(10):893-95.
[10] Awadallah SM, Atoum MF, Nimer NA, Saleh SA. Ischemia modified albumin: an oxidative stress marker in β-thalassemia major. *Clin Chim Acta*. 2012;413 (9-10):907-10.
[11] Mehmetoglu I, Yerlikaya FH, Kurban S, Erdem SS, Tonbul Z. Oxidative stress markers in haemodialysis and peritoneal dialysis patients, including coenzyme Q10 and ischemia-modified albumin. *Int J Artif Organs*. 2012;35(3):226-32.
[12] Mahapatra SK, Das S, Dey SK, Roy S. Smoking induced oxidative stress in serum and neutrophil of the university students. *Al Ameen J Med Sci*. 2008;1(1):20-31.
[13] Burlakova EB, Zhizhina GP, Gurevich SM, Fatkullina LD, Kozachenko AI, Nagler LG, et al. Biomarkers of oxidative stress and smoking in cancer patients. *J Cancer Res Ther*. 2010;6(1):47-53.
[14] Sinha MK, Gaze DC, Tippins JR, Collinson PO, Kaski JC. Ischemia modified albumin is a sensitive marker of myocardial ischemia after percutaneous coronary intervention. *Circulation*. 2003;107:2403-05.
[15] Lountzis NI, Ferringe T, Macaron N, Amy Howard. Oral Submucous Fibrosis. http://emedicine.medscape.com/article/1077241-overview.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Mar 10, 2015**
Date of Peer Review: **Jun 10, 2015**
Date of Acceptance: **Aug 12, 2015**
Date of Publishing: **Nov 01, 2015**