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ORIGINAL RESEARCH

Implications Of Smoking And Gutkha Chewing On Salivary Vitamin B12 And Folic Acid Levels In Chronic Periodontitis

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Abstract

Background: Periodontitis is a commonly occurring oral inflammatory disorder and often has a multifactorial etiology. Lack of proper oral hygiene, inadequate nutrition and use of tobacco consumption are some of the common causative factors of periodontitis.

Aim of the study: Estimation and correlation of salivary vitamin B12 (VB12) and folic acid (FA) levels in individuals with different periodontal status.

Materials and Methods: A total of 120 participants, aged 18-60 were selected for the study. The study population was divided into four subgroups of 30 participants each based on clinical condition of periodontium and habits of smoking and gutkha chewing. The periodontal parameters evaluated for the study participants included, gingival index (GI) probing pocket depth (PPD) and clinical attachment level (CAL). Spectrophotometric analysis was performed to evaluate VB12 and FA levels from saliva samples of the participants.

Results: Smokers demonstrated highest VB12 levels than the other groups while FA levels were decreased among both smokers as well as gutkha chewers. The preriodontal parameters were significantly raised in all the other groups in comparison to healthy controls.

Conclusion: The present study, investigated the possible association between the habits of gutkha chewing and smoking with the salivary levels of vitamin B12 and FA in chronic periodontitis subjects. The higher VB12 and lower FA levels evidenced in this study reflect increased periodontal inflammation among smokers and gutkha chewers; thus highlighting the deterioration of periodontal tissue caused by these habits.

Keywords: Periodontal inflammation, Chronic periodontitis, Cigarette smoking, Folic acid, Gutkha chewing, Healthy Periodontium,Vitamin B12

INTRODUCTION

Chronic periodontitis (CP) is a commonly occurring gum disorder. The prevalence of chronic preiodontitis increases with age and is almost 89.6% in the 35-44 age range [1].Literature attributes a wide range of diverse of contributing factors for development of CP; these include bacterial infections resulting from bacterial infections, side effects from drugs, hormonal variations, social, behavioral, systemic, environmental and genetic risk elements[2] .Tobacco is commonly extracted from two main species: Nicotina tabacum and Nicotina rustica and contains the addictive agent nicotine. Nicotine creates a stimulant effect on all organs, but chiefly binds to central nervous system receptors and increases the dopamine levels in brain. Guthka, a commonly used preparation of smokeless tobacco includes several harmful constituents such as N-nitrosonornicotine (NNN) [3] and carcinogens like N-nitrosamines [4].

Localized gingival recession (25-30%) is one of the most prevalent tobacco induced oral changes [5]. Literature states that cigarette smoke poorly alters the VB12 levels due to presence of cyanide [6].Further, interactions of tetrahydrofolic acid with cyanate in cigarette smoke leads to folate deficiency in periodontal tissues [7].

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Other than oral cancers and potentially malignant lesions smoking and smokeless tobacco cause several other oral, especially gingival and periodontal diseases. Empirical literature states that more than one-half of the cases of adult periodontitis can be attributed to cigarette smoking [8]; thus highlighting the extensive damage caused by this destructive addiction on periodontal health. Previous researches have demonstrated almost 6 times higher prevalence of periodontal tissue destruction among smokers [9]. Similarly, smokeless tobacco users demonstrate significantly higher prevalence of destructive oral changes such as gingival and periodontal clinical attachment loss (CAL), recession, furcation involvement in the multi-rooted teeth, and different types of periodontal diseases [10].

It has been proven that cigarette smoking alters the VB12 and FA metabolism [11] .VB12 is a water soluble vitamin that acts as a cofactor in metabolism and in RNA and DNA synthesis, making it important for production of new cells during development and healing. [11,12]. FA (also known as folate or vitamin B_9), is also a water soluble vitamin, that is necessary for nucleotide biosynthesis. Factors affecting VB12 and FA are listed in [Figure 1]. Adequate levels of FA are essential for maintenance of periodontal tissue health. FA is particularly important during periods of rapid cell division and growth of the squamous epithelium; and its deficiency can lead to gingival and periodontal necrosis, loss of periodontal ligament and alveolar bone height [2, 12].

Previous studies have generated very limited evidence regarding salivary VB12 and FA levels among guthka chewers. This study aimed to assess and compare the levels of VB12 and FA in saliva of CP patients with two of the most common tobacco consumption habits in India, i.e. smoking and gutkha chewing.

MATERIALS AND METHODS

Study Design 120 systemically healthy participants, between the age of 18-60 years, were enrolled randomly from the out-patient department of the P.M.N.M Dental College and Hospital, Bagalkot. Pregnant and lactating females, individuals with history of trauma, acute /chronic systemic disorders, recent tooth extraction or history of periodontal treatment, antibiotics or anti-inflammatory therapy, 3 months before the start of this study were excluded.

Written informed and signed consent was taken from all the participants after duly explaining them with the need and design of the study. Based on the condition of periodontium and tobacco use habit history, the study population was further divided into four subgroups with 30 participants each [n=30]. Group I included individuals with clinically healthy periodontium, Group II included individuals having CP, gutkha chewers having CP were clubed in Group III while smokers with CP constituted Group IV.

Group I participants had healthy periodontium with no signs of inflammation, while Group II participants had >30 percent of the dentition affected with periodontitis, the participant of these two groups did not consume tobacco. Group III included only the regular users of chewable smokeless tobacco, (at least 1 sachet daily for at least 12 months prior to the study period) [13]. Current smokers and individuals with history of smoking ≥100 cigarettes in their lifetime were included in Group IV [14].

Periodontal Parameters

The periodontal parameters evaluated for this study included, gingival index (GI), probing pocket depth (PPD) and clinical attachment level (CAL). To reduce any possibility of inter-examiner variability and bias, only one examiner was engaged in obtaining all the measurements by using a standardised procedure [15]. Group I included individuals with no signs of clinical gingival inflammation (GI=0) or clinical attachment loss (CAL=0), the probing pocket depth was less than 3 mm (PPD ≤ 3 mm). Group II participants had at least 30% sites where the probing pocket depth was (PPD ≥ 5 mm), gingival inflammation was (GI >1mm) and clinical attachment loss (CAL ≥4 mm). Group III and Group IV included participants with gutkha chewing and smoking habits respectively with CP.

Saliva sampling

Participants were asked to abstain from eating or drinking one hour before collection of their respective saliva samples. The tobacco users were instructed to refrain from smoking/tobacco chewing one hour prior to saliva collection. The samples were collected in a quiet room between 9am to 11:30 am to prevent any cardiac variations. The participants rinsed their mouths using distilled water. The stimulated saliva was collected for at least 5 minutes in Eppendrof tube and kept at low temperature [16]. The saliva samples were centrifuged to remove cell debris for 5 minutes at 3000 rpm.

Spectrophotometric analysis: Standardized method was used to obtain calibration curve for VB12 and FA [Figure 2]. The saliva sample was mixed with equal parts of distilled water in a test tube. This solution was forcedly shaken and vibrated for 30 minutes and 10 minutes respectively. A UV-visible spectrophotometer (Shimadzu Model) was set at 361 nm wavelength to measure the levels of VB12 and FA [17].

Statistical analysis

The Statistical Package for Social Sciences (SPSS) software (version11) was used for the analysis of the study results. Mean and standard deviation values of the observed parameters were calculated for results. The Pearson correlation coefficient test was used to evaluate the study parameters among the four groups while the Kruskal Wallis test and Mann-Whitney U test were employed to measure the pair-wise

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differences between the groups. The p value at $p \leq 0.5$ was considered as statistically significant.

RESULTS

Group II, III and IV demonstrated significantly higher mean values ($p< 0.001$) of the evaluated gingival and periodontal parameters (GI, PPD and CAL) in comparison to Group I. The pair wise comparison of these parameters of Group I with other groups using the Mann-Whitney U test was statistically significant ($p <0.001$).

Upon performing the pair wise comparison, statistically significant difference in mean values of VB12 was observed on comparing Group I (p=0.000), Group II (p=0.000) and Group III (p=0.001) with Group IV. The levels of VB12 were observed to be significantly higher in group IV than the other three groups. The mean levels of FA also demonstrated statistically significant differences upon pair wise comparison (p = 0.000), with lower concentrations being noted for smokers [Table 1]. Mean values of salivary VB12 & FA levels and clinical scores (GI, PPD, CAL) in each of the four study group has been given in [Table 2].

Table 1: Pair-wise comparison of study groups by Mann-Whitney U-Test for VB12 and FA levels

Primary Group		Pair wise comparison with other groups	V B12 (p value)	FA (p value)
Group I	**v/s**	Group II	.264	.014
		Group III	.008	.000
		Group IV	.000	.290
Group II	**v/s**	Group III	.117	.084
		Group IV	.000	.000
Group III	**v/s**	Group IV	.001	.000

Significant results for VB12 - Group I/II v/s Group IV and for FA - Group III v/s Group I/II

Table 2: Association between Vitamin B12, FA levels, GI, PPD, CAL score in each group.

Groups	Vit B12 & FA (Mean)	GI Score				PPD Score				CAL Score				
		0	1	2	3	0	5	6	7	0	5	6	7	8
Group I	Vit B12	18.9	18.36	-	-	18.50	-	-	-	18.50	-	-	-	-
	FA	3.53	3.68	-	-	3.64	-	-	-	3.64	-	-	-	-
Group II	Vit B12	-	27.91	24.80	14.85	-	29.52	19.86	21.90	-	25.77	27.78	18.02	23.60
	FA	-	2.54	2.61	3.73	-	2.46	2.84	3.47	-	2.19	2.76	2.66	3.74
Group III	Vit B12	-	26.73	31.28	76.50	-	26.00	39.57	7.60	-	21.69	34.81	38.88	7.60
	FA	-	2.27	2.11	0.90	-	2.33	1.93	1.63	-	2.42	2.01	2.15	1.63
Group IV	Vit B12	-	45.54	50.18	56.55	-	47.89	56.05	-	-	57.87	37.40	59.62	-
	FA	-	4.41	4.06	3.74	-	4.27	3.50	-	-	5.08	3.79	3.73	-

Abbreviation - GI, gingival index; PPD, pocket probing depth; CAL, clinical attachment level; VB12, vitamin B-12; FA, folic acid.

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Correlation between GI, PPD, Vitamin B12 and Folic Acid

In Group I, the r value of GI for FA (r <0.066) and VB12 (r <0.062) was statistically insignificant. The co-relation of GI with VB12 and FA was statistically significant and directly proportional in Group III; whereas it was inversely proportional for Group IV. PPD was inversely proportional but significantly related (p = 0.037) with FA for Group III; while in Group IV, a significant co-relation was observed between PPD and VB12 (p=0.047).

Table 3: Correlation between Vitamin B12 and FA within study groups

Study Groups	Pearson correlation value
Group I	.053
Group II	.037
Group III	-.014*
Group IV	.305

*(-) shows negative correlation

DISCUSSION

Literature demonstrates negative influence of smoking and chewing tobacco on nutrition levels, host immune response and integrity of hard and soft tissues of the oral mucosa [12].The present study investigated the possible association between smokeless tobacco and smoking with the salivary levels of vitamins such as vitamin B12 and FA in chronic periodontitis subjects. It was observed that VB12 levels were highest in Group IV while the FA levels were lowest in Group III. Exposure to cigarette smoke may result in chemical inactivation and deficiency of FA [18].

It has been recognised that smokers exhibit poor diet and lower intake sources of FA, VB12 and vitamin B6 [2,11,19] . Earlier literature demonstrates that smokers include a greater proportion of statured fats and comparatively lesser uptakes of antioxidant, vitamins and fibre in their diets [20].

The results of this study for saliva are similar with an earlier work, reporting these parameters in serum [11]. Both salivary FA and VB12 values were noted to be elevated in gutkha chewers than in smokers. This may be attributable to actual physical contact of smokeless tobacco with the outer alveolar tissue, and reaction of by-products of gutkha, such as cyanide; that may lead to elevated levels of VB12. The correlation of participant's age and CAL with VB12 and FA was statistically insignificant in all the four groups. The findings of this study concur with previous researches, highlighting variations in the values of FA and VB12 among tobacco using CP patients.

CONCLUSION

This study provides empirical confirmation that gutkha chewing and smoking, alters the salivary VB12 and FA concentrations and thus, increases the periodontal inflammation. Relatively small sample size of this study has generated promising results and further investigations with larger sample sizes are strongly encouraged to obtain and confirm more precise characterization of investigated antioxidants as biomarkers.

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Conflicts of interest There are no conflicts of interest

REFERENCES

1. Shaju JP, Zade RM, Das M. Prevalence of periodontitis in the Indian population: A literature review. J Indian Soc Periodontol. 2011; 15(1):29-34.
2. B Sumona, S Sheetal, M Anil, P Suvarna. Comparative evaluation of serum folic acid levels in smokers and non-smokers with chronic periodontitis. Bangladesh Journal of Medical Science. 2011; 10(2):83-90.
3. Muthukrishnan A & Warnakulasuriya S. Oral health consequences of smokeless tobacco use. Indian J Med Res. 2018; 148(1):35-40.
4. Walsh P M, Epstein J B. The Oral Effects of Smokeless Tobacco. J Can Dent Assoc. 2000; 66(1):22-5.
5. Johnson GK, Slach NA. Impact of Tobacco Use on Periodontal Status. J Dent Educ. 200;65(4):313-321
6. Piyathilake CJ, Macaluso M, Hine RJ, Richards EW, Krumdieck CL. Local and Systemic effects of cigarette smoking on folate and vitamin B12. Am J Clin Nutr. 1994; 60(4):559–66.
7. Francis KT, Thompson R W, Krumdieck C L. Reaction of tetrahydrofolic acid with cyanate from urea solutions. Formation of an inactive folate derivative. Am J Clin Nutr. 1977; 30(12):2028–32.
8. Fisher M A, Taylor G W, Tilashalski KR. Smokeless tobacco and severe active periodontal disease, NHANES III. J Dent Res. 2005; 84(8):705-10.
9. Guentsch A et al. Lipid peroxidation and antioxidant activity in saliva of periodontitis patients: effect of smoking and periodontal treatment. Clin Oral Invest. 2008;12(4):345–35.
10. Singh GP, Rizvi I, Gupta V, and Bains VK. Influence of smokeless tobacco on periodontal health status in local population of north india : a cross-sectional study. Dent Res J (Isfahan). 2011; 8(4):211–20.
11. Erdemir EO, Bergstrom J. Relationship between smoking and folic acid, vitamin B12 and some haematological variables in patients with chronic periodontal disease. J Clin Periodontol. 2006;33(12):878–84.
12. George JP, Shobha R, Lazarus FJ. Folic acid: A positive influence on periodontal tissues during health and disease. Int J Health Allied Sci. 2013;2(3):145-52.
13. Periodontal conditions and oral symptoms in gutka-chewers with and without type 2 diabetes. Acta Odontologica Scandinavica. 2008;66(5):268-273.

DOI: 10.69605/ijlbpr_14.4.2025.8

14. Tomar S L, Asma S. Smoking - attributable periodontits in the United States: Findings from NHANES III. J Periodontol. 2000;71(5):743-75.
15. Loe H, Silness J. Periodontal disease in pregnancy. Acta Odont. Scand. 1963;21:533-51
16. Patel S P, Raju P A. Resistin in serum and gingival crevicular fluid as marker of periodontal inflammation and its correlation with single nucleotide polymorphism in human resistin gene at −420. Contemp Clin Dent. 2013;4(2):192–7.
17. Karmi O , Zayed A , Baraghethi S , Qadi M , Ghanem R. Measurement of vitamin b12 concentration: a review on available methods. The IIOAB Journal. 2011;2(2):23-32.
18. Rungsunn et al. Relationship of tobacco smoking with serum vitamin B12, folic acid and haematological indices in healthy adults. Public Health Nutr. 2003;6(7):675–81.
19. Giraud DW, Martin HD, Driskell JA. Erythrocyte and plasma B-6 vitamer concentrations of long-term tobacco smokers, chewers, and nonusers. Am J Clin Nutr. 1995; 62(1):104–9.
20. Dallongeville J, Marceaux N, Fruchart JC, Amouyel P. Cigarette smoking is associated with unhealthy patterns of nutrient intake: a meta-analysis. J Nutr. 1998; 128(9):1450–7.