Determining the Effect of Gutkha on Serum Levels of Vitamin B12 and Folic Acid as Compared to Smoking among Chronic Periodontitis Subjects: A Cross-Sectional Study

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ABSTRACT

Background: Periodontitis, being a common inflammatory disease has a multifactorial origin, with smoking and gutkha as few of the causative entities. The role of smoking as a risk factor for periodontitis is well documented in literature. Cigarette smoke also affects vitamin B12 and folic acid mechanisms. Nutritionally derived vitamin B12 occurs mainly as either hydroxycobalamin or deoxyadenosycobalamin. Folic acid is also heat sensitive and water soluble, closely linked to vitamin B12 in its metabolism. However, effect of smokeless tobacco in form of gutkha on serum levels of vitamin B12 and folic acid is yet to be explored.

Aims and Objectives: To estimate and correlate serum vitamin B12 (VB12) and folic acid (FA) levels among periodontally healthy subjects and Chronic Periodontitis (CP) subjects with habit of smoking and gutkha chewing.

Materials and Methods: The study included 111 subjects ranging in age from 18 to 60 y. Participants were divided into four groups: 30 healthy subjects (Group I), 29 subjects with CP (Group II), 25 smokers with CP (Group III) and 27 gutkha chewers with CP (Group IV). Clinical parameters included pocket probing depth (PPD), clinical attachment level (CAL) & gingival index (GI) following which VB12 and FA levels were estimated through UV-spectrophotometry method and data was analysed using Statistical Package for Social Scientists software, Mann-Whitney U-test and Pearson correlation coefficient. p-values less than 0.05 were considered as significant.

Results: Pairwise comparison by Mann-Whitney U-test showed an increase in the serum VB12 in Group IV when compared to Group I (p=0.01) and Group II (p=0.01). Although serum FA levels were found to be low in Group III (7.61 ug/ml) & Group IV (8.64 ug/ml), Group III was found to be statistically significant (P=0.046). The clinical parameters GI, PPD and CAL among the four groups of patients were also statistically significant (p < 0.05).

Conclusion: The study results suggested that among the patients with periodontal disease, serum VB12 levels are directly related while serum FA levels are inversely related to inflammation and tissue destruction in periodontium as occurred in Group IV.

Keywords: Chronic periodontitis, Folic acid, Gutkha, Smoking, Vitamin B12

INTRODUCTION

Biochemical and microbiological research has suggested that periodontal disease is the result of an interplay between bacterial activity and the host tissue [1]. The host response to gingival microorganisms is characterized in part by an influx of polymorphonuclear leukocytes or neutrophils (PMN) [2]. Periodontitis is a multifactorial disease, manifestation and progression of which is influenced by a wide variety of determinants, including social, behavioral, systemic, environmental and genetic risk factors [3]. Cigarette smoking is a well established risk factor for periodontitis and is associated with an increased risk for periodontal attachment loss and bone loss [4]. The biological plausibility of the increased periodontal disease severity and rate of progression associated with smoking has been hypothesized to be due to interactions among smoking, bacterial periodontal pathogens, and the host [5].

Exposure to environmental cigarette smoke is associated with increased leucocyte counts, chemotaxis an increased release of reactive oxidants from stimulated neutrophils. Smokers demonstrate 2.6 – 6 times increased prevalence of periodontal diseases compared to non-smokers [6]. Cigarette smoking also affects VB12 and FA mechanisms [7].

Smokeless tobacco has also been shown to affect the immune response both in vitro and in vivo [5]. Clinical attachment level (CAL), gingival recession, mobility, furcation and lesions like leukoplakia, periodontal disease, delayed wound healing and dental caries were significantly higher amongst smokeless tobacco users as compared to those not consuming smokeless tobacco [8]. Although available in many different forms, smokeless tobacco is mainly used as chewing tobacco and snuff (moist or dry) [9].

VB12 is a water-soluble, heat-sensitive vitamin of the B-vitamin group. It is often protein bound as methylcobalamin, hydroxycobalamin and deoxyadenosycobalamin in nutrients [7]. FA (also known as folate) is an essential vitamin, which is also heat sensitive and water soluble. It belongs to the class of vitamin compounds related to pteroylglutamic acid (PGA), which serve as cofactors in the enzymatic transfer of single carbon units in a variety of metabolic pathways [10]. Factors affecting VB12 and FA are listed in [Table/Fig-1].

Folate deficiency, which is associated with increased oxidative stress, endothelial dysfunction, genomic instability, defective DNA repair, and apoptosis, has been shown to be related to a number of human diseases, including periodontal disease [3]. Repair and maintenance of periodontal tissue requires high turnover of squamous epithelium which is impaired in cases of reduced levels of FA. Deficiency of FA is also known to cause necrosis of gingiva, periodontal ligament and loss of alveolar bone [10].
Cyanide, being one of the major component of cigarette smoke, adversely affects VB12 nutritional status [11]. Further tetrahydrofolic acid combines with cyanate to yield a biologically inactive compound [12], which could result in folate deficiency in tissues affected by cigarette smoke. Organic nitrates also inactivate methyl cobalamin by cleaving the methyl–cobalamin bond [13].

The chemical carcinogens in smokeless tobacco include polynuclear aromatic hydrocarbons (usually benzo-a-pyrene), polonium-210, and N-nitrosamines. Other chemicals include radium-226 and lead-210 [14]. The primary periodontal alteration in smokeless tobacco users is localized gingival recession (25-30 %) [15]. Whereas the untoward effect of smoking on periodontal health is abundantly documented, little is known about the possible effects of non smoked tobacco products [9] and it’s effect on serum levels of VB12 and FA. To our knowledge from the indexed literature, no study has yet analysed the comparative evaluation of serum VB12 and FA concentrations in patients with chronic periodontitis who are Gutkha users.

Taking this into consideration, the present study was conducted to estimate and correlate the serum VB12 and FA levels among periodontally healthy (Group I), chronic periodontitis (Group II) subjects, smokers with CP (Group III) & gutkha chewers with CP (Group IV) and also to correlate the serum levels with clinical parameters which will be of great importance in the diagnostic and preventive strategy of periodontal diseases.

**MATERIALS AND METHODS**

**Study Design**

In the present cross-sectional study, 120 systemically healthy subjects aged between 18 to 60 y were randomly selected from the out-patient department, Department of Oral medicine, P.M.N.M Dental College and Hospital, Bagalkot, India among which nine were excluded (four aggressive, three noncurrent smoker and two on steroid therapy for OSMF ). Further, all the participants were clearly explained regarding the need and design of the study. Written informed consent was obtained from all recruits. The research project was approved by the Ethical Committee.

The selection of patients was made according to the criteria approved by the 1999 International Workshop for the classification of periodontal diseases and conditions [16]. Further, in Department of Periodontics,111 Patients were divided into four groups as clinically healthy periodontium (Group I), chronic periodontitis (Group II), smokers with CP (Group III) & gutkha chewers with CP (Group IV).

All the study participants with no history of any acute/chronic systemic disorders were included. Subjects belonging to the group III were enrolled if they had smoked ≥100 cigarettes in their lifetime and currently smoked [17]. Gutkha chewers with chronic periodontitis (Group IV) were enrolled if they regularly chewed smokeless tobacco at least one sachet daily for atleast 12 months [18]. Pregnant women, lactating mother, individuals with trauma or who underwent recent tooth extraction or who had received any periodontal / antimicrobial and anti-inflammatory therapy or vitamin supplements in last three months before sampling were excluded from the study.

**Periodontal parameters**

Depending upon the GI, PPD and CAL measurements, study subjects were divided into 4 groups:

- **Group I (n= 30)**: Periodontally healthy subjects characterized by GI=0 (absence of clinical inflammation), PPD ≤ 3 mm and CAL=0,
- **Group II (n= 29)**: Subjects with CP characterized by at least 30% sites with PPD ≥ 5 mm, GI >1 and CAL ≥4 mm,
The present cross sectional study was carried out among 111 subjects and categorized into 4 groups based on their history and clinical presentation. Pairwise comparison of all the subjects revealed the absence of female subjects in Group III and Group IV and comparatively younger subjects in Group IV [Table/Fig-4]. The differences between the four groups were significant (p< 0.001) in terms of GI scores, PPD and CAL [Table/Fig-4]. The mean scores of all the parameters (GI, PPD, CAL) were significantly higher among Group II, III and IV compared to Group I (p < 0.001). Likewise, pairwise comparison of GI, PPD and CAL among four groups by Mann-Whitney U-test were highly significant (p<0.001). The mean GI score was statistically significant when Group II was compared with Group III (p< 0.004) [Table/Fig-5].

Upon pairwise comparison of Four Groups, significant difference in mean VB12 levels were found between Group I and Group IV (p= 0.01) and Group II and Group IV (p= 0.01) [Table/Fig-6]. The VB12 values were significantly higher in group IV as compared to other groups. Likewise, significant difference in mean levels of FA was found between Group I and Group III, (p = 0.046) and Group II and Group III (p=0.009) suggesting it's concentration being lower in gutkha chewers compared to other groups. The mean FA score was statistically significant when Group II was compared with Group III (p< 0.004) [Table/Fig-5].

The statistical analysis was performed using Statistical Package for Social Scientists (SPSS) software (version11). The results were presented as mean and standard deviation. Any differences between the four groups along with the clinical and serum variables, viz. GI, PPD,CAL,VB12 and FA were carried out through Pearson correlation coefficient test . The pair-wise differences among the four groups were carried through Mann-Whitney U-test and Kruskal Wallis test. The p-value i.e. p ≤ 0.5 was considered to be statistically significant.
Correlation between GI, PPD, Vitamin B12 and Folic Acid

In group I, the r-value of GI in relation to FA and VB12 is positively correlated (r < 0.066) and (0.062). However, when seen statistically, results are insignificant. In group III, GI is positively and significantly correlated with FA and VB12 i.e. if GI increases, FA and VB12 will increase. In group IV, GI is negatively correlated with FA and VB12 but p<0.05 in relation to FA. In group III, PPD is negatively correlated and P-value is significant (p = 0.037) in relation to FA. When comparing PPD with VB12, it is found significant (p=0.047) in group IV [Table/Fig-8].

DISCUSSION

Smoking and smokeless tobacco have shown to impair various aspects of innate and immune host responses. The habit of chewing tobacco is increasing because of its free availability and cheaper cost and ban on smoking at public places in India. Adults currently using smokeless tobacco are twice as likely to have severe active periodontal disease than adults who never used smokeless tobacco [5]. Although there is wide documentation of the adverse effects of cigarette smoking on periodontal disease, vitamin B12 and folic acid but the effects of gutkha on concentrations of water soluble vitamins is less well studied.

Cobalamin is released from intrinsic factor cobalamin complex and binds either to transcobalamin (T C II) or serum haptocorins. TC II accounts for 10-30 % of the measurable serum cobalamin level. After absorption of folate in jejunum, the principal storage site is the liver. Distribution of folate to other tissues depends mostly on enterohepatic recirculation, in which folate in a methylated form is re-absorbed from bile into the serum [24]. Folic acid works with vitamin B12 in many functional processes throughout the body, including the peroxidation [7].

Methylcobalamin is essential for the removal of the methyl group from 5-methyl tetrahydrofolate in the homocysteine - methionine transmethylation catalyzed by methionine synthase [Table/Fig-9]. As a consequence, vitamin B12 deficiency or inactivation results in most of the folates being ‘trapped’ in a form (5-methyl tetrahydrofolate) that cannot be utilized. Folate deficiency secondary to the “methyl folate trap” could therefore develop in the vitamin B- 12 depleted tissues [11].

Interestingly, the results suggested that VB12 levels were highest in Group IV as compared to other groups while FA levels were found to be decreased in Group III and Group IV when compared to healthy and CP groups. In group IV, there is also a risk of lesions like oral submucous fibrosis that causes restricted mouth opening in later stages and which can be a reason of less intake of fruits and vegetables leading to decreased levels of folic acid as compared to group I and group II. The serum folic acid levels of smokers was less in comparison with that of healthy subjects which is in accordance with several previous studies [11,25,26].

Via chemical inactivation, exposure to cigarette smoke may result in folic acid deficiency that principally affects the bronchial epithelium, rendering it more susceptible to neoplastic transformation by the carcinogenic hydrocarbons of tobacco smoke [26].

Smoking stimulates the oxidative burst of neutrophils, increases reactive oxygen species production and leads to lipid peroxidation and alterations in protein carbonyls in plasma [4]. Erdemir EO and Bergstro J have investigated the relationship between smoking and the serum levels of FA, VB12 in patients with periodontal disease. The serum FA concentration of smokers was lower than that of non-smokers (p < 0.05) whereas serum VB12 concentrations did not significantly differ between the two groups [7].

Rungus et al., have reported that the serum FA of smokers was lower than that of nonsmokers which was not significantly different and serum VB12 levels were significantly higher in smokers than the non-smokers which supports the results of our study [26].

CONCLUSION

Among the risk factors, smoking and chewing tobacco are the most prevalent ones which are also the most common oral abusive habits in today’s life style. Our study provides evidence that gutkha being one of the causative factors for periodontitis, indeed showed difference in serum levels of VB12 and FA, contributing to the inflammatory burden on periodontium. We suggest that higher VB12 and lower FA levels reflect increased periodontal inflammation.

However, the present study suggests that the impact of quantified tobacco use on periodontitis in small population may
be limited to the importance of serum VB12 and FA levels and, the cardinal role of oral hygiene as one of the aetiology of periodontal disease should also be assessed.

Special educative methods should be designed depicting the role of gutkha on systemic health other than oral cancer. Further intervention with expanded study sample including other fluids is acceptable to be more specific towards the effect of gutkha which can set these two vitamins as biomarkers for oral health in future. It becomes important to consider gutkha chewing which can be as harmful as smoking for periodontitis.

REFERENCES