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"ASSESSMENT OF LIVER ENZYMES, TOTAL SERUM PROTEINS AND PERIODONTAL STATUS IN GHUTKA CHEWERS AND ORAL SUBMUCOUS FIBROSIS PATIENTS."

Dental Science

Dr. Vijayalaxmi Kokatnur — Reader, Department Of Periodontology, P.M.N.M. Dental College, Bagalkot, Karnataka 587103.

Dr. Haziel Diana Jenifer* — Reader, Department Of Periodontology, Yenepoya Dental College, Mangalore- 575018. *Corresponding Author

Dr. Subramaniam Rao — Professor, Department of Periodontology, P.M.N.M Dental College, Bagalkot , Karnataka 587103.

Dr. Madhur Manak — Scientist D (Medical), ICMR, Delhi Headquarters 110029.

ABSTRACT

INTRODUCTION: Ghutka is a form of smokeless tobacco with a mixture of tobacco and areca nut. Addictive consumption of ghutka can give rise to a premalignant condition called Oral Submucous Fibrosis (OSMF). Arecolin, a major constituent of areca nut, disrupts the harmony of periodontal cells and leads to destruction of the periodontium, and also shown to have some deleterious effects on the liver. Hence, the aim of this study was to assess and correlate the levels of serum Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and total serum proteins (TSP) and periodontal status in ghutka chewers (GC) and oral submucous fibrosis patients (OSMF). **MATERIALS AND METHODS:** The study comprised of 90 subjects. Group I= 30 Chronic Periodontitis (CP), Group II= 30 ghutka chewers with chronic periodontitis without OSMF (CP+GC) and Group III= 30 ghutka chewers with chronic periodontitis and OSMF (CP+GC+OSMF). Clinical parameters like gingival index, plaque index, probing pocket depth and clinical attachment loss were recorded. Biochemical assessment of serum AST, ALT and TSP levels were carried out through UV absorption spectrophotometry. ANOVA, Mann-Whitney U-test and Newman-Keuls post-hoc procedure were used for statistical analysis. **RESULTS:** Significantly higher levels of serum AST, ALT enzymes and TSP levels (P=0.000) and greater periodontal destruction were seen among OSMF subjects. Results showed positive correlation with number of packs/day and biochemical parameters. **CONCLUSION:** Higher levels of periodontal breakdown and significant variation in liver enzymes and total serum proteins were appreciable in subjects with habit of ghutka chewing and OSMF as compared to those without habit.

KEYWORDS

Alanine Aminotransferase, Aspartate Aminotransferase, Chronic Periodontitis, Oral Submucous Fibrosis

INTRODUCTION

Smokeless tobacco and arecanut consumptions are widespread around the globe including some parts of U.S. Ghutka is mainly an addictive consumption found in South Asian regions of the world. Ghutka is the form of smokeless tobacco consisting of chewable agents, primarily betel nuts (Areca catechu) which contain substances like arecoline, an active alkaloid found in betel nuts, stimulating fibroblasts to increase the production of collagen by 150% and 2.04gm of nicotine/100gm of the product, which on testing showed pH levels sufficient to contribute to the quick absorption of nicotine in the blood, delivering to the customer the desired kick.[1] Oral submucous fibrosis is one the most prevalent and premalignant condition caused by tobacco use affecting not only the oral cavity, but also the oropharynx.[2] The term derives from oral (mouth), submucosal (below the mucosa of the mouth), and fibrosis (meaning hardening and scarring).[3] Although the onset of OSMF is insidious, there is unquestionably a need to thoroughly evaluate biological status of an individual. The prevalence rate of OSMF is 0.5% with possibility rate of 7.6-13% of malignant transformation, affecting mostly the persons aged 20-40 years and a incidence of 29.094 yrs and male: female ratio of 34:15.[4]

Periodontitis being a multifactorial and chronic inflammatory disease, prevalent among adults affecting the periodontium..[5]Tobacco in smoke or smokeless form along with the local bacterial residence is considered as significant environmental risk factors for periodontitis. Studies have shown the impact of ghutka on periodontium wherein, the loss of clinical attachment level along with local gingival recession at the site of placement was appreciated.[6,7]

The Arecolin that is present in areca nut is known to have some deleterious effects on liver functioning. Higher concentrations of transaminase enzymes, i.e aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are present in liver, and muscles. These enzyme elevations in blood indicate liver function impairment and this in turn may have an effect on collagen regeneration, and hyalinization of collagen tissues. Singh N evaluated serum SGPT (ALT) levels in 150 patients and observed raised levels in all the cases and suggested that role of systemic involvement in OSMF cannot be ignored.[8] However a strong association was also demonstrated between periodontal active sites and presence of high levels of gingival crevicular fluid AST: thus supporting the view that AST levels of serum is an indicator of tissue destruction.[9,10] Oral submucous fibrosis is characterized by definite increase in fibrous collagen, which is protein in nature. Some of the studies have found a correlation between total serum protein (TSP) levels and degree of OSMF.

Thus the present study was undertaken to assess and compare the serum AST, ALT and TSP levels in ghutka chewers with chronic periodontitis and oral submucous fibrosis.

MATERIALS AND METHODS

Source of data

In the present cross-sectional bio-chemical study was conducted in the Department of Periodontology, PMNM Dental College and Hospital, Bagalkot, Karnataka, India. Around 96 systemically healthy subjects were screened for potential participation, among which 90 subjects between 23 and 50 years of age were selected after estimation of the sample size . Further, 6 subjects were excluded (4 on steroid therapy for OSMF, 2 systemically unhealthy) . Subjects belonged to the group of patients referred to outpatient Department of Periodontics of P.M.N.M Dental College, Bagalkot, Karnataka, for the treatment of chronic periodontitis and were selected randomly. The distribution of 90 subjects according to age and gender distribution is shown in **Table 1**.

The study was approved by the Ethical Committee of the Institution. The subjects were divided into 3 groups: Group I – 30 chronic periodontitis (CP), Group II: 30 Ghutka chewers with chronic periodontitis (GC+CP) without OSMF and Group III: 30 Ghutka chewers with chronic periodontitis and OSMF (GC+CP+OSMF).

The inclusion criteria for patients with chronic periodontitis were probing pocket depth (PPD) ≥ 4mm, clinical attachment level (CAL) ≥ 3mm, and habit of chewing ghutka for at least 3 years >5 times per day.[11]

OSMF was assessed only clinically through inspection and palpation adhering to the clinical classification given by Ramanathan et al. [12] Histological examination was not possible as maximum participants

didn't agree for biopsy. The clinical examination was carried out by the periodontists along with the oral medicine staff. All the stages of OSMF were considered as OSMF present or absent with no further staging categorization.

Patients with any systemic disease, allergies or drug usage, smokers, history of periodontal treatment in the past 6 months, or pregnant and lactating women, and with any history of liver, muscle, heart, and kidney diseases and on any medication which are known to affect SGPT and SGOT and protein levels were excluded from the study.

A thorough medical and dental history was obtained, followed by a complete oral and clinical examination. The procedure was clearly explained to patients and informed consent was obtained. The study was in accordance with the Helsinki Declaration of 1975 as revised in 2002.

Clinical assessment: Clinical periodontal parameters, including Plaque index (PI), Gingival index (GI), Probing depth (PPD) and Clinical attachment loss (CAL) were recorded as shown in **Table 2**.

Blood sample collection: Around 2ml of blood was drawn from the anti-cubital fossa &immediately transferred to the K3EDTA tubes.

Spectrophotometric analysis of serum

Biochemical analysis of serum was carried out in Department of Pharmacology, HSK College of Pharmacy, Bagalkot, India. The serum was separated from blood by centrifugation at 3000-3500 rpm for 15 min. Biochemical analysis of SGPT & SGOT was assessed through the Kinetic method and total protein through Biuret method. Supernatant samples of both were assessed by UV spectrophotometer (Beckman DU640) set at 600nm wavelength to calculate the values through absorbance.

Statistical analysis

Data was analyzed using Statistical Package for Social Sciences version 12 (SPSS Inc. Chicago,USA 1999),. The results were presented as mean and standard deviation. Overall differences among the three groups for all variables including GI, PI, PPD, CAL, ALT, AST and TSP were determined by ANOVA. The pairwise differences among the three groups were carried by Mann Whitney U test and Newman Keuls post hoc procedure. The $P < 0.05$ was considered to be statistically significant.

RESULTS

This cross-sectional study included total of 90 subjects divided into three groups. Group I, Group II and Group III [**Table 1**]. The intergroup comparison of periodontal and serum parameters were carried out by ANOVA test [**Table 2 and 3**]. The differences between the three groups were significant ($P < 0.001$) in terms of PI, PPD, CAL, ALT, AST and TSP scores. The mean ALT, AST and TSP score was statistically significant when Group I was compared with Group II ($P < 0.05$). Comparison of mean GI scores among all 3 groups showed no significant result. Whereas pairwise comparison between Groups I and III, and Groups II and III showed a highly significant result among all the parameters except the GI ($P=0.901$).

Likewise, pairwise comparisons of PD and CAL among the three groups were highly significant ($P < 0.001$) [Table 2]. A significant difference in mean serum ALT, AST and TSP levels were observed over all study groups ($P < 0.001$) [**Table 4**]. Mean values of ALT, AST and TSP were found to be below the normal range in group I as compared to Group II and III with a significant P value ($P< 0.001$). The mean PI score was highest in Group III (2.18±0.66) with statistically significant P value ($P< 0.001$). [Table 2]

On comparison and evaluation of all the parameters with the amount of ghutka packs/day a subject consumes, carried out through Pearsons correlation, revealed a highly significant positive correlation with plaque index (0.404) followed by duration (0.418) and other serum parameters (Table 5).

DISCUSSION

Areca nut extracts present in Ghutka have shown to have a significant causative role in initiating periodontal diseases along with the variables such as oral hygiene levels, dietary factors, and general dental health status.[13, 14] Ghutka chewing interferes with the microbial mechanism of neutrophils, and inhibits protein synthesis and attachment of fibroblasts. This in turn promotes bacterial colonization and periodontal infection. As a result, the harmony between various periodontal structures is disrupted that leads to induced gingivitis and periodontitis.[14]

The result of our study strongly supports this causal relationship of ghutka chewing and progressive periodontal destruction. The Plaque Index, probing pocket depth and clinical attachment loss were comparatively greater in ghutka chewers (Group II and III) than non-chewers (Group I). Patients with OSMF showed higher rate of periodontal destruction as compared to those without the lesion. This may be attributed to the restricted mouth opening and inadequate oral hygiene maintenance coupled with destruction of periodontal tissues by the toxic substance present in Ghutka.

The results of the present study were in accordance with those of Akhter *et al*.[15] who reported that betel quid chewers exhibited higher mean PPD and CAL. In their study, mean PPD was approximately 3.8 ± 0.7 mm and CAL was 4.2 ± 1.2 mm in betel quid chewers. Besides, Ling *et al*.[16] had also found strong positive relation between habit of betel quid chewing and severity of periodontal destruction. Dodani *et al*. had reported PPD in patients with OSMF and without OSMF as 1.88 ± 0.36 and 1.68 ± 0.16 mm, respectively.[17] Chu *et al*. had also reported PPD of 1.8 ± 0.38 mm and CAL of 0.52 ± 0.76 mm in betel quid chewers.[18]

Continued consumption of Ghutka is a well-known causative factor for the development of the premalignant condition, Oral submucous fibrosis. Arecolin, a chief constituent of Ghutka has shown to cause some deleterious effects on the liver. And hence we evaluated the liver enzymes, serum AST and ALT in these individuals and found that these enzymes levels were significantly higher in subjects with OSMF as compared to other two groups. This is in accordance with the study carried out by Chandy et al, [19]where in effect of arecanut usage on liver was assessed and found that the liver enzymes were progressively higher in these individuals.

Our study also aimed to find out any relevance with regard to serum protein in subjects with OSMF. Fibrous collagen is biochemically protein in nature (procollagen) similar to protein of other cells as suggested by Van Wyk et al.[20] OSMF characterized by definite increase of collagen deposition which is a protein, in the areas affected by this condition locally. According to a study by Goel et al., there is a definite alteration in plasma amino acid level in OSMF patients as compared to control.[21] Our study was in accordance with a study by Sannad et al, where in a definite correlation was observed between the total serum protein levels and higher grades of OSMF owing to increase in total serum protein with progressive OSMF.[22] Similar result was seen in our study.

Some studies have demonstrated that the severity of periodontal disease appears to be related to the duration of tobacco use and amount of daily tobacco intake. [23] This is in correlation with the present study where in the amount of packs /day, and the duration of ghutka chewing with significant increase in plaque index is well confirmed.

CONCLUSION

As ghutka chewing is harmful, the alarming scenario demands that health agencies and governmental organizations to launch awareness programs to inform and educate the public regarding the adverse health consequences and possible cancer risk associated with tobacco consumption.

Estimation of liver enzymes like aspartate aminotransferases and alanine aminotransferases and total serum proteins may serve as a useful tool in determining the degree of pre malignancy and also periodontal breakdown status in subjects with the deleterious habit of ghutka chewing and oral submucous fibrosis.

Table 1: Mean and SD values of age, and gender distribution among the study groups.

Groups	N	Age(yrs) mean± SD	M: Fe ratio (%)
I	30	34.3±8.11	70:30
II	30	32.8±11.21	80:20
III	30	31.9±10.4	73:27

Table 2: Mean and SD values of clinical parameters among the study groups

Group	PI	GI	PPD(mm)	CAL(mm)
I	0.91±0.41	1.72±0.61	5.59±0.42	4.46±1.43
II	1.2±0.48	1.34±0.6	6.75±0.38	5.5±1.40
III	2.18±0.66	1.7±0.64	6.98±0.38	5.17±1.32
P value	<0.000*	<0.153	<0.022*	<0.012*

GI: Gingival index, PPD: Pocket Probing depth, CAL: Clinical attachment levels *statistically significant ($P < 0.05$)

Table 3: Mean and SD values of ALT, AST and TSP among the study groups

Group	ALT (U/L)	AST (U/L)	TSP mg/l
I	4.11±1.79	2.50±1.46	58.14±27.9
II	7.40±5.25	10.43±6.40	121.3±18.54
III	12.8±8.81	15.41±7.88	142.46±19.01
P value	<0.0000*	<0.000*	<0.000*

ALT:Alanine aminotransferase , AST: Aspartateaminotransferase , TSP: Total protein.*statistically significant ($P < 0.05$), U/L: International units per litre

Table 4: Pairwise comparison of mean periodontal and serum parameters

	GI	PI	PPD	CAL	ALT	AST	TSP
Group I vs Group II	0.081	0.081	0.217	0.967	0.041	0.000*	0.000*
Group I vs Group III	0.901	0.000*	0.008*	0.015*	0.000*	0.000*	0.000*
Group II vs Group III	0.117	0.000*	0.100	0.009*	0.016*	0.108	0.003*

GI: Gingival index, PPD: Pocket Probing depth, CAL: Clinical attachment levels, ALT:Alanine aminotransferase , AST: Aspartateaminotransferase, TSP: Total protein.*statistically significant ($P < 0.05$).

Table 5: Pearsons correlation of Packs/day consumption with the clinical and serum parameters.

	Age	D	GI	PI	PPD	CAL	ALT	AST	TSP
Packs/day	-.118	0.418**	-.178	0.404**	0.247	-.133	0.509**	0.617**	0.704**

D: Duration, GI: Gingival index, PPD: Pocket Probing depth, CAL: Clinical attachment levels, ALT:Alanine aminotransferase , AST: Aspartateaminotransferase , TSP: Total protein.

REFERENCES

1. Canniff JP, Harvey W. The aetiology of oral submucous fibrosis: the stimulation of collagen synthesis by extracts of areca nut. Int J Oral Surg 1981; 10:163-7.
2. Passi D, Bhanot P, Kacker D, Chahal D, Atri M, Panwar Y. Oral submucous fibrosis: newer proposed classification with critical updates in pathogenesis and management strategies. Natl J Maxillofac Surg. 2017;8(2):89. doi: 10.4103/njms.NJMS_32_17
3. Mishra G, Ranganathan K. Matrix metalloproteinase-1 expression in oral submucous fibrosis: An immunohistochemical study. Indian J Dent Res 2010;21:320-5
4. Kanthem RK, Guttikonda VR. Serum lipid profile in oral submucous fibrosis: A clinico pathological study. J Oral MaxillofacPathol 2015;19:139-44.
5. Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4:1-6.
6. Robertson PB, Walsh M, Greene J, Ernster V, Grady D, Hauck W. Periodontal effects associated with the use of smokeless tobacco. J Periodontol 1990; 61:438-43.
7. Fisher MA, Taylor GW, Tilashalski KR. Smokeless tobacco and severe active periodontal disease, NHANES III. J Dent Res 2005; 84:705-10.
8. Singh N.,SinghJ.,Singh U: Oral submucous fibrosis- a new approach to treatment with combined therapy J Indian Dent Assoc 1996; 67: 168-170.
9. Persson RG, De Raven TA, Page RC: Relationship between levels of aspartate aminotransferase in gingival crevicular fluid and gingival inflammation. J Periodontal Res 1990; 25: 17- 24.
10. Rajini, Mehta DS:Comparison of AST levels in GCF before and after periodontal Phase I therapy. Using Pocket watch. J Indian Dent Assoc 1999;72: 70-5.
11. Ahmad MS, Ali S A, Ali A S, Chaubey K K. Epidemiological and etiological study of oral submucous fibrosis among gutkha chewers of Patna, Bihar, India. J Indian Soc Pedod Prev Dent 2006;24:84-9.
12. Ramanathan K. OSMF-An alternative hypothesis as to its causes. Med J Malaysia 1981;36:243-5
13. Parmar G, Sangwan P, Vashi P, Kulkarni P, Kumar S. Effect of chewing a mixture of areca nut and tobacco on periodontal tissues and oral hygiene status. J Oral Sci. 2008;50:57–62.
14. Rathod S, Wanikar I, Raj A, Maske S, Harkare V. Association between kharra chewing and periodontal health status in oral submucous fibrosis patients of Central India, Nagpur. J Indian SocPeriodontol 2018;22:345-7
15. Akhter R, Hassan NM, Aida J, Takinami S, Morita M. Relationship between betel quid additives and established periodontitis among Bangladeshi subjects. J ClinPeriodontol 2008;35:9-15.
16. Ling LJ, Hung SL, Tseng SC, Chen YT, Chi LY, Wu KM, et al. Association between betel quid chewing, periodontal status and periodontal pathogens. Oral MicrobiolImmunol 2001;16:364-9.
17. Dodani K, Anumala N, Avula H, Reddy K, Varre S, Kalakonda BB, et al. Periodontal findings in patients with oral submucous fibrosis and comet assay of affected gingival epithelial cells. J Periodontol 2012;83:1038-47.
18. Chu YH, Tatakis DN, Wee AG. Smokeless tobacco use and periodontal health in a rural male population. J Periodontol 2010;81:848-54
19. Chandy ML, Kannan N, Patil R, Mathew V, Mohamed A, Sreeja PK, Jose R. Assessment of Alteration in High Density Lipo Protein, Apolipoprotein A1, Serum Glutamic Pyruvic Transaminase and Serum Glutamic Oxaloacetic Transaminase in Oral Submucous Fibrosis Patients. World Academy of Science, Engineering and Technology International Journal of Medical and Health Sciences Vol:10, No:5, 2016.
20. Van Wyk CW. Oral Submucous Fibrosis. The South African experience. Indian J Dent Restorat 1997;8:39-45.
21. Goel R, Chandrashekhar T, Ramani P, Sherlin JH, Natesan A, Premkumar P. Amino Acid Profile in Oral Submucous Fibrosis: A High Performance Liquid Chromatography (HPLC) Study. J ClinDiagn Res. 2014 Dec; 8(12): ZC44–ZC48.
22. Sannad A, Tamgadge S, Tamgadge A, Yadav KS, Giri A, Wankhede M. Total serum protein estimation and its correlation with clinical and histopathological grading using Masson's Trichrome stain in patients of oral submucous fibrosis. ContempClin Dent 2017;8:286-92.
23. Palmer R, Soory M. Modifying factors: Diabetes, puberty, pregnancy and the menopause and smoking. In: Lindhe J, Karring T, Lang P, editors. Clinical Periodontology and Implant dentistry. 4th ed 2003. P. 188.