Research Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2020;10:2s:1-7.

Assessment of Serum Levels of Vitamin A and Vitamin E in Oral Submucous Fibrosis Patients

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ABSTRACT

Background: Oral submucous fibrosis is a common precancerous condition. The primary factor considered in the pathogenesis of OSMF is the frequent use of betel nut. However, several other factors are believed to contribute to the development of OSMF including nutritional and vitamin deficiencies. There have been very few studies to determine the nutritional aspect of this insidious, chronic disease. Hence this study is carried out to estimate the serum vitamin A and vitamin E levels in OSMF patients and to compare with the healthy controls.

Methods: A case-control study was conducted on 20 cases of OSMF and 20 controls. The serum vitamin A and vitamin E of all 40 participants were estimated by using a spectrophotometer.

Results: The mean Vitamin A value in the patient group was calculated to be 26.30 µg/dl whereas, in the control group, it was 29.45 µg/dl. Comparing the values between different stages, there was a gradual reduction in the Vitamin A levels as the stage advanced. The mean Vitamin E value in the patient group was 7.57 µg/dl which was marginally lower than the mean Vitamin E levels in the control group (8.08 µg/dl). Comparing the values between different stages, there were almost similar values of the Vitamin E levels across all the stages.

Conclusion: The serum levels of vitamin A and vitamin E between subjects with OSMF and the healthy controls did not show any significant difference when statistically evaluated. Nor was there any considerable difference across different stages of the disease. Therefore, we could not arrive at any evidence for vitamin deficiency among this population of OSMF patients. However, studies involving larger samples and more accurate methods are suggested in the future.

Keywords: Betel nut, Oral submucous fibrosis, Vitamin A, Vitamin E.

INTRODUCTION

The oral mucous membrane is a unique area of the body, which is continuously exposed to various kinds of stresses such as heat, cold, microorganisms, chemicals and mechanical irritants. In response to these stresses, both epithelial and connective tissue layers of oral mucosa exhibit acute and chronic reactive changes. One such reaction to areca nut of the collagen of oral mucosa is Oral submucous fibrosis.[1] of all the collagen disorders affecting man oral submucous fibrosis is of particular interest to dentists since we are predominantly concerned with the orofacial structures. It is approximately half a century since Schwartz described oral submucous fibrosis in the tobacco-chewing women of Indian origin in Kenya. Since then, this condition has evoked an intense enthusiasm among many researchers in India and throughout the world. Various authors had investigated the condition

Received: Dec. 5, 2019**: Accepted**: Jan. 27, 2020
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pISSN 2278-0076
eISSN 2321-9270

thoroughly and proposed several factors that play a role in the etiopathogenesis of this condition. Current evidence suggests that arecoline in the areca nut is the key factor in initiating the disease process.[2-5] This condition is aptly described by Pindborg and Sirsat as "an insidious, chronic disease affecting any part of the oral cavity and sometimes the pharynx. Although occasionally preceded by and/or associated with vesicle formation, it is always associated with a juxta-epithelial inflammatory reaction followed by a fibroelastic change of the lamina propria, with epithelial atrophy leading to stiffness of the oral mucosa and causing trismus and inability to eat.[6,7] The habit of betel quid chewing is widespread throughout India and South East Asia. This condition is also reported in Asian immigrants living in other parts of the world. Various researchers have conducted studies in different parts of the country to find out the incidence of betel quid chewing habit in the general population. At present, this habit is widely prevalent in teenagers and young adults and gaining social acceptance. Oral submucous fibrosis predominantly involves the oral cavity. The buccal mucosa, retromolar area, and the soft palate are the predominantly affected sites. The mucosa in the involved areas gradually becomes pale, followed by progressive stiffness of subepithelial tissues. In addition to the involvement of oral mucosa, this condition also involves the pharynx and oesophagus especially in persons who chew and swallow the products of betel quid.[8,9] The malignant transformation of OSF is reported to be between 2.3 and 7.6 %. The most ironical aspect of this condition is the lack of appropriate and satisfactory treatment modalities and lack of case-control studies to show evidence of other contributing factors in the development and progression of fibrosis. The underlying mechanisms by which areca quid ingredients induce OSF and oral cancer have been under intensive investigation. Areca nut extract is cytotoxic to gingival keratinocytes, oral mucosal fibroblasts and epithelial cells. In addition, the activity of areca nut ingredients to induce reactive oxygen species (ROS) and genetic damage in oral cells has been well documented. Notably, an increased ROS level in the oral cavity of volunteers chewing areca quid and the induction of oxidative DNA damage in the hamster's buccal pouch exposed to areca nut extracts have been demonstrated. Collectively, these results indicate a crucial role of ROS in areca-induced cytotoxic effects. The antioxidant activities help to protect the cells from potential damage by free radicals and ROS. Antioxidant defence system includes specific vitamins like vitamin A, vitamin C, vitamin E and certain minerals such as selenium, copper, zinc and manganese.[10-12]It is proved to be beneficial in patients with cardiovascular diseases, diabetes mellitus, and inflammatory diseases and for the prevention of chronic processes such as atherosclerosis and carcinogenesis. A number of studies found that low intake of vitamins was associated with increased risk for carcinogenesis. Gupta Soma et al. in 2004 reported that the plasma vitamin E levels were decreased significantly in OSMF patients with respect to healthy controls and the decrease in Vitamin E was found to be more significant in OSMF grade 2 and grade 3 than grade 1. In their study, patients reportedly showed an increase in plasma levels of vitamins after 6 weeks of oral administration of the same and an associated clinical improvement was reported.[13-16]There is an overwhelming response from the dentists to supplement OSF patients with vitamin preparations and antioxidants. However, there have been very few studies to determine the nutritional aspect of this chronic insidious disease. Hence a case-control study was carried out to estimate the serum vitamin A and vitamin E levels in OSMF patients and to determine their implications in the pathogenesis of OSF.

The present study was done with an aim to estimate the serum levels of vitamin A and vitamin E in oral submucous fibrosis patients.

MATERIALS AND METHODS

A case-control study was carried out in 20 patients who visited the dental clinic of the Department of Oral Medicine and Radiology and oral pathology and were clinically diagnosed and histopathologically confirmed cases of oral submucous fibrosis. Selection of cases was on the basis of the ore of the following characteristics as suggested by from a workshop held in Kuala Lumpur consensus Malaysia, reported by Zain et al. (1996): Palpable fibrous bands, The mucosal texture feels tough and leathery and Blanching of the mucosa together with histopathological features characteristic of OSF

Inclusion criteria were: Age group 18 to 65 years, clinically and histopathologically confirmed cases of OSF and Patients free from any systemic disease Exclusion Criteria: Patients with co-existing other oral mucosal disorders, Patients taking any form of vitamin therapy, Patients who have already taken any treatment for oral submucous fibrosis. All the enrolled subjects were then interviewed and examined in the dental chair in the dental clinic using clinical examination tools and recorded in case sheet proforma. All the details of the patients like the name, age, sex, occupation and address were logged. The history of chewing habit (betel nut, pan, gutkha) was noted along with duration and frequency. The selected patients were divided into three clinical stages as mentioned by Bailoor DN. and Nagesh (2001) Incisional biopsy was carried out under local anaesthesia after obtaining patient consent. The tissue specimens were subjected to histopathological evaluation. 3 ml of venous blood was obtained by venipuncture of the median cubital vein under aseptic precautions. The blood samples were collected in sterile containers and sent for serum vitamin A and vitamin E estimation. Vitamin A was estimated using photoelectric calorimetry and vitamin E by calorimetry, both using spectrophotometer systronics spectrophotometer-106.

Statistical analysis

The data was coded and entered into Microsoft Excel spreadsheet. The analysis was done using SPSS version 15 (SPSS Inc. Chicago, IL, USA) Windows software program. The variables were assessed for normality using the Kolmogorov-Smirnov test. Descriptive statistics were calculated.

RESULTS

The study group: 20 histopathologically confirmed oral submucous fibrosis cases, subdivided into 3 stages based on clinical presentation.

In the study group, the age of the subjects ranged from 20 to 55 years. The majority (60%) of these cases were within 26-35 years, followed by below 25 years (20%), (Table1) The mean age was 31.6 years. Males comprised 85% of the study group (17/20), while females formed the remaining 15% of the group (3/20). The control group also consisting of patients with similar age and sex distribution Based on the clinical staging of the cases, 30% of cases belonged to stage1, stage 2 formed 40% and 30 % of cases were in stage3 (Table 2). All the patients in the study group were habitual betel chewers; the most common type of chewing involved processed betel nut (Table 3). Around 40% of the patients performed the chewing 5 times or less per day. 40% of patients had the habit for a period of 5 years or less, 40% chewed for 6 to 10 years and 20% for more than 10 years. When statistical comparison using unpaired t-test was made between the study group and the controls for the serum vitamin A levels, statistical difference was not significant (p=0.197) (Table 4). The values between the stages of OSF were assessed using ANOVA test and found no meaning in spite of showing considerable difference (P=0.102). (Table 5) The statistical comparison made between the study group and the controls for the serum vitamin E levels using unpaired t-test; the statistical difference was not significant (p=0.094) (Table.6). The values between the stages of OSF were also not significant (p= 0.562) by ANOVA (Table 7).

An attempt was made to compare the clinical staging with the histopathological grading using the data. Statistical comparison was made to see the relation of the clinical-stage with the total duration of the chewing habit performed. The Chi-square test showed a statistically significant association (P=.004) A statistical comparison was also made to see the relationship of the histological grading with the total duration of the chewing habit performed. The Chi-square test showed a statistically significant relationship (P=.017).

Table 1: Age Distribution.

Age group [in years]	Total	Percentage
<25	4	20%
26–35	12	60%
36–45	1	5%
>46	3	15%

Table 2: Stage Wise Distribution of Cases.

STAGE	NO. OF CASES	PERCENTAGE
STAGE 1	6	30%
STAGE 2	8	40%
STAGE 3	6	30%

Table 3: Chewing Habits.

Habit	Total	Percentage
Raw betelnut	1	5%
Local pan	6	30%
Processed betelnut	13	65%

Table 4; Serum Level of Vitamin-A between Cases & Controls (Unpaired T-Test) Group Statistics.

	group	N	Mean	Std Deviation	t	P-value
Vitamin A (µg/dl)	Cases	20	26.30	8.962		
					-1.312	0.197(ns)
	Controls	20	29.45	5.907		

Table 5: Serum Level of Vitamin-A In Various Stages (ANOVA).

	Stages	N		Mean	Std. Deviation	F	P-value
Vitamin A	Stage1		6	32.67	8.802		
	Stage2		8	24.37	7.945	2.619	0.102
	Stage3		6	22.50	8.216		

Table 6: Serum Level of Vitamin-E Between Cases & Controls (UNPAIRED T-TEST)

	group	N	Mean	Std .	t	P-value
Vitamin E (µg/dl)						
	Cases	20	7.575	0.9118		
	Controls	20	8.085	0.9664	-1.717	0.094(ns)

Table 7: Serum Level of Vitamin-E In Various Stages (ANOVA).

	Stages	N	Mean	Std . Deviation	F	P-value
Vitamin E	Stage1	6	7.783	0.5947		
	Stage2	8	7.675	0.8276	0.6	0.562 (ns)
	Stage3	6	7.233	1.2801		

DISCUSSION

The strong association between areca nut chewing and oral submucous fibrosis is known for a long time. In addition to the cytotoxic effects of areca nut extracts, the activity of areca nut ingredients to induce reactive oxygen species (ROS) and genetic damage in oral cells has been well documented [17]

In this study, an attempt was made to analyze the serum vitamin A and vitamin E levels in oral submucous fibrosis patients and compared with normal healthy individuals, thereby explaining the oxidative stress. An attempt was also made to differentiate the same between different stages of the disease.

Analyzing the results of our study, it can be noted that the patients were within the age range of 20 to 55 years, with a mean age of 31.6 years. Ranganathan K et al. (2004)[18] reported a mean age of 32.4 years. The gender distribution in the present study for the patients and the control group was 5.6:1(male: female) that was close to the observation of 6:1 by Kiran K et al. (2007)[19], 4.9:1 by Hazarey VK et al. (2007)[20] and 4.6:1 by Ariyawardana A et al. (2006)[21]

All the patients in our study had at least any one of the areca nut-based chewing habits. Similar observations were made in more extensive studies by Shear et al. (1967)[22] Dockrat & Shear (1969)[23], van wyk CW et al. (1990)[24] and various others. It has been identified as the most important etiological factor. Some workers also noticed OSMF in patients without any oral habit, e.g. 1.81% by Shah N (1998)[25], 0.12% by Gupta S (2004)[1] et al., but none in our study. Among the types of betel products, majority of the patients (65%) chewed processed betel nut commercially available in the form of paan Parag, star, Maruthi etc. Kiran K et al. (2007) [19] found 81% and Ranganathan K et al. (2004)[18] found 71 % of patients chewed paan masala and Shah et al. (1998)[25] reported that paan masala chewing produced OSF changes in a shorter period of time than betel quid chewing.

Based on the clinical staging, 30% of patients belonged to stage 1, 40% stage 2 and 30 % in stage 3. Haider SM et al. (2000)[26] and Raina C et al. (2005)[27]observed that more than half of patients and Hazarey VK et al. (2007)[59] found almost half of the patients in their study were in clinical stage 3. However, their staging criteria were different.

The mean serum vitamin A levels in the patient group were 26.30 µg/dl and the controls 29.45 µg/dl. Metkari SB et al. (2007)[28] found vitamin A level 18.22 µg/dl in the patients compared to 37.85 in controls. It is to be noted that there was a reduction of vitamin A levels in patients compared to controls in our study, but it was not statistically significant (P=0.197). Similarly, a gradual reduction in the vitamin A levels was found as the clinical stage progressed, but not evident statistically (P= 0.102).

Comparing the vitamin E levels between patients and controls, only a marginal difference was found, i.e. 7.57µg/dl in patients against 8.05µg/dl in controls which was statistically non-significant (P= 0.094). Comparing the same between the three stages also found a marginal difference that was not significant. Gupta S et al. (2004)[1] observed plasma beta carotene and vitamin E levels decreased significantly in OSF patients with respect to healthy controls. The decrease in beta-carotene and vitamin E was found to be more significant in OSMF grade II and III than in grade I. They also proceeded with oral administration of beta-carotene and vitamin E, which resulted in an increase in plasma level of these two antioxidants and associated clinical improvement of OSF. As β-carotene is a provitamin-A carotenoid that is efficiently converted to vitamin A than other carotenoids, it can be directly compared to vitamin A.

Thomas G et al. (2003)[29] have reported that high intake of fruits and vegetables can act as a protective shield for OSMF , which again upholds the theory that poor nutrition is one of the causative factors of OSMF .

The observations are not sufficient to establish an etiologic or contributory role for nutritional deficiencies in OSF. It is probable that the lack of these factors observed among OSF patients in few studies may be secondary; most OSF patients cannot tolerate spicy food, which is a healthy family and community diet in the Indian subcontinent and the opening of the mouth in OSF patients becomes progressively smaller.

In this study, an attempt was made to compare the clinical staging with the histopathological grading. The analysis showed no significant difference between the two systems followed. Or in other words, the clinical stage is comparable to the histological grading in most cases. However, histopathological examination shows localized tissue changes whereas OSF is a well-recognized precancerous condition that is more often generalized, affecting various parts of the oral cavity to a different extent; sometimes even pharynx and oesophagus may be involved.

A comparison was also made to observe the relationship of the clinical staging and the histological grading with the total duration of the chewing habit performed. It was seen that both the systems had a positive relationship with the length of the chewing practice. Sinor PN et al. (1990)[30] made a similar observation that both the duration and the frequency of chewing increased the relative risk. Shah N, Sharma PP (1998)[25] found that duration of chewing was not significantly correlated but the rate of chewing was directly co-related to the manifestation of OSF. They explain that exposure to the total burden of various harmful substances in a given time was more significant than the entire duration of exposure. Maher R et al. (1994)[31] from Pakistan also made a similar observation that the daily consumption rate appeared to be much more significant with respect to risk than the lifetime duration of the habit.

CONCLUSION

The serum levels of vitamin A and vitamin E between subjects with OSMF and the healthy controls did not show any significant difference when statistically evaluated. Nor was there any considerable difference across different stages of the disease. Therefore, we could not arrive at any evidence for vitamin deficiency among this population of OSMF patients. However, studies involving larger samples and more accurate methods are suggested in the future.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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