

## Original Article

## ASSOCIATION BETWEEN ORAL SQUAMOUS CELL CARCINOMA AND SERUM VITAMIN E IN SNUFF USERS AND NON SNUFF USERS IN KHYBER PAKHTUNKHWA PROVINCE OF PAKISTAN

\*Abbas Saleem Khan, \*\*Sajjad Ahmad, \*\*\*Zahoorullah, \*\*\*\*Muhammad Rauf Khattak,  
\*\*\*\*\*Muhammad Mumtaz Khan

\*Department of Oral Pathology Peshawar Dental College, Riphah International University.

\*\*Department of Pathology, Peshawar Medical College, Riphah International University.

\*\*\*Department of Biochemistry, Peshawar Medical College, Riphah International University.

\*\*\*\*Department of IRNUM (Institute of Radiotherapy and Nuclear Medicine), University campus, Peshawar

\*\*\*\*\*Department of Pathology Peshawar Medical College, Riphah International University.

### ABSTRACT

**Objective:** To assess the association between oral squamous cell carcinoma (OSCC) and serum vitamin E status in snuff users and nonusers.

**Materials and Methods:** This comparative cross-sectional study was conducted in Peshawar Medical and Dental College and Institute of Radiotherapy and Nuclear Medicine (IRNUM) Peshawar. This study was carried out from March 2013 to August 2013. The 50 diagnosed cases of oral squamous cell carcinoma and 50 healthy controls were selected by purposive sampling technique. Among the oral squamous cell carcinoma cases, 25 subjects (group A) were snuffed users, and 25 subjects (group B) were non-snuff users. Among the healthy controls, 25 participants (group C) were snuff users, and 25 participants (group D) were non-snuff users. Blood samples were collected from all 100 participants. Serum vitamin E levels were estimated spectrophotometrically.

**Results:** Negative association (OR=0.85;  $p$ -value=0.838) was observed between oral squamous cell carcinoma and low serum vitamin E status. Negative (OR=0.02) but statistically significant association ( $p$ -value=0.0036) was found between serum vitamin E status and well-differentiated squamous cell carcinoma only. Positive (OR=2.37) but statistically insignificant ( $p$ =0.24) association was observed between vitamin E status and snuff usage among healthy controls.

**Conclusion:** There is no positive association between OSCC with low serum vitamin E status amongst snuff dippers and non-snuff dippers.

**Keywords:** Oral squamous cell carcinoma, vitamin, Snuff.

### INTRODUCTION

Oral epithelial malignancy constitutes a significant component of the global cancer burden.<sup>1,2</sup> Oral squamous cell carcinoma (OSCC) is the most frequent histological variant among all oral epithelial malig-

nancies (90 – 95 % ).<sup>1</sup> The disease is almost equally prevalent in all the provinces of Pakistan.<sup>3-7</sup> According to collective cancer registry report (Dec.1994-Dec.2015) of Shaukat Khanum Memorial Cancer Hospital and Research Center (SKMCH & RC), it is the third most common malignant tumor among all age groups in both the sexes; ranked second among adults in both the genders ; and marked first among adult male and third commonest malignant tumor in adult female population.<sup>8</sup> The etiology of OSCC is multifactorial.<sup>1,9</sup> Among the common predisposing

#### Correspondence:

**Dr. Abbas Saleem Khan**

Assistant Professor, Oral Pathology Peshawar Dental College,  
Warsak Road Riphah International University-Islamabad Peshawar,  
Khyber Pakhtunkhwa, Pakistan

Cell: 03339161379

Email address: abbassaleem@gmail.com

factors are; usage of intraoral tobacco products (both smoked and smokeless),<sup>10-12</sup> alcohol consumption,<sup>13,14</sup> chronic infection by oncogenic viruses; [Human Papillomavirus (HPV) , Herpes simplex Virus (HSV)]<sup>1</sup>, oral potentially malignant disorders,<sup>15,16</sup> use of alcohol-containing mouthwashes,<sup>17</sup> poor socioeconomic conditions coupled with poor oral hygiene,<sup>1,18</sup> familial predisposition,<sup>1,19</sup> and intake of a diet lacking important nutrients which have a protective role against the development of malignancies.<sup>20</sup>

Various forms of smokeless tobacco (naswar or snuff, pan/betel quid, gutka, khaini, mishri, Zarda, nass, mawa, Shammah and tombak) are consumed in many parts of the world including Pakistan, India and other South East Asian countries.<sup>1,21-24</sup> Snuff or naswar (a mixture of crushed tobacco, powdered lime, ash, indigo, cardamom oil and menthol) is extensively used in various parts of Pakistan, predominantly in Khyber Pakhtunkhwa province.<sup>24-26</sup> All forms of tobacco are carcinogenic and are strongly associated with the causation of oral squamous cell carcinoma. Smokeless tobacco contains 28 carcinogens.<sup>27,28</sup> The most harmful carcinogens are tobacco-specific nitrosamines (TSNA). They include four compounds, among them two, N-nitrosornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone or Nicotine-derived nitrosamine ketone (NNK) are considered to be potentially carcinogenic.<sup>29,30</sup> These carcinogenic chemicals damage the DNA of squamous cells of epithelial lining of oral cavity resulting in mutation of genome of these cells and ultimately leading to development of malignant lesions of the affected area.<sup>31</sup>

There exists a relationship between diet and cancer development.<sup>32,33</sup> Experimental studies in animals and clinical studies in humans have shown that antioxidant vitamins, such as E, C and A; may inhibit cancer formation and progression. The anticancerous properties of vitamin E comprise of anticarcinogenic and immunostimulatory effects. Vitamin E is the most effective antioxidant. It acts as the first line of defense against lipid peroxidation by protecting polyunsaturated fatty acids in the cell membrane through its free radical interception activity at an early stage of their action. In addition to its role as a free radical scavenger, vitamin E also enhances the body's immune response against tumor development.<sup>34-36</sup> Epidemiological studies have observed a relationship between vitamin E levels and risk of various malignancies; e.g, oral squamous cell carcinoma,<sup>37,38</sup> esophageal squamous

cell carcinoma,<sup>39</sup> carcinoma lung,<sup>40</sup> and breast<sup>41</sup>.

In Pakistan and particularly in the province of Khyber Pakhtunkhwa, there is a scarcity of study on the association of oral squamous cell carcinoma with serum vitamin E status among snuff users and non-snuff users has been published.

The present cross-sectional study was designed to compare serum vitamin E levels among OSCC cases and healthy controls, and to determine the possible association of OSCC and its histological grades with serum vitamin E status in snuff users and non-snuff users to validate the role of vitamin E in the etiology of oral cancer.

## MATERIALS AND METHODS

This study was conducted from March 2013 to August 2013 at Peshawar Medical and Dental College and Institute of Radiotherapy and Nuclear Medicine (IRNUM), having a total of 100 participants.

The study group consisted of histologically confirmed cases with and without a history of oral snuff use (n=50) and age, sex, and habit matched healthy individuals as controls (n=50). They were further subdivided into four groups on the basis of exposure to snuff use ((Group A: OSCC subjects exposed to snuff, Group B: OSCC subjects not exposed to snuff, Group C & D: healthy controls exposed and not exposed to snuff; respectively). Nonprobability; purposive sampling technique was used. Subjects were selected from the outdoor patient department (OPD) of the participating centers. From the selected individuals; with co-existing other oral mucosal lesions, alcohol users, cigarette smokers and taking vitamin E supplements and therapy were excluded from the study. A detailed history of cases and controls was recorded on a structured proforma designed for the study.

For serum vitamin E levels; 5 ml of venous blood was taken (after an overnight fast) from all study participants in plain (red topped) vacutainer. The blood was allowed to clot and centrifuged at 3000 rpm for five mins, and then serum was aspirated through micropipettes and placed in serum cups and stored at -20 °C till analyzed. Serum vitamin E levels were measured by a spectrophotometric method of Nair and Magar<sup>42</sup> using spectrophotometer (Model MS-4375 China) against the standard. According to this method serum, vitamin E level less than 0.361mg/dl was considered as low level and the status was marked as deficient

vitamin E status while serum vitamin E level equal to or more than 0.361 mg/dl was considered as normal serum vitamin E levels. The study was conducted after approval by Institutional Review Board (IRB) of Peshawar Medical College and permission from Director office, IRNUM. Those who were willing to participate in the study were included, and informed consent was taken. The data obtained were analyzed using statistical package for social sciences (SPSS) version 19. Results were given as mean and standard deviation (SD) for continuous variables (age, serum vitamin E levels). Difference between the two groups for vitamin E status was analyzed for statistical significance using Chi-square test and Fisher's exact test where appropriate. Single sample t-test was used to measure the statistical significance of mean of age and serum vitamin E levels for all the groups. Serum vitamin E levels between the two groups were compared by using Student's t-test. Association between different variables was assessed by calculating Odds' ratio. P value for the precision of odd's ratio was also calculated. A probability value of less than and equal to 0.05 ( $p \leq 0.05$ ) was considered statistically significant. Odd's ratio of less than one shows a negative association and more than one

indicates a positive relationship.

**RESULTS**

The 50 diagnosed cases of oral squamous cell carcinoma and 50 healthy controls were selected. Among the oral squamous cell carcinoma cases, 25 subjects (group A) were snuff users, and 25 subjects (group B) were non-snuff users while healthy controls, 25 participants (group C) were snuff users and 25 participants (group D) were non-snuff users.

The age of carcinoma cases ranged from 30-70 years with mean age of 52yrs. Male to female ratio was 4:1 among carcinoma cases.

Statistically, a non-significant difference was observed for a total period of snuff use ( $p$ -value=1), the quantity of snuff used ( $p$ -value=0.699) and current snuff usage status ( $p$ -value=0) among patients and controls with the history of snuff usage. The most common site of development of oral squamous cell carcinoma was tongue (32%) in both snuff users and nonusers followed by the floor of the mouth (24%) and palate (22%). The most common histological grade was MDSCC (moderately differentiated squamous cell

**Table 1: Study subjects characteristics and vitamin E status**

VARIABLES	OSCC cases				Healthy controls				TOTAL		p-value
	Exposed to snuff		Not exposed to snuff		Exposed to snuff		Not exposed to snuff				
	N	%	N	%	N	%	N	%	N	%	
A) Gender	25	25	25	25	25	25	25	25	100	100	0.317
i) Male	18	18	23	23	24	24	15	15	80	80	
ii) Female	7	7	2	2	1	1	10	10	20	20	
<b>B) Pattern of snuff use</b>											
<b>i) Present status</b>											
Current snuff user	8	16	-	-	25	50	-	-	33	66	0.734
Ex snuff user	17	34	-	-	0	0	-	-	17	34	
<b>ii) Frequency of snuff used</b>											
Less than to 5times/day	3	6	-	-	5	10	-	-	8	16	0.701
More than 5 times/day	22	44	-	-	20	40	-	-	42	84	
<b>iii) Exposure period to snuff</b>											
Less than 10 years	9	18	-	-	8	16	-	-	17	34	1.765
More than 10 years	16	32	-	-	17	34	-	-	33	66	
<b>iv) Habit of snuff ingestion</b>											
Yes	2	4	-	-	1	2	-	-	3	6	0.617
No	23	46	-	-	24	48	-	-	47	94	
<b>C) Tumor extension</b>											
Localized	24	48	19	32	-	-	-	-	43	86	0.098
Lymph node metastasis	1	2	6	12	-	-	-	-	7	14	
<b>D) Vitamin E status</b>											
Deficient	11	11	18	18	18	18	13	13	60	60	0.838
Normal	14	14	7	7	7	7	12	12	40	40	

\*Chi-square and Fisher exact test were applied where applicable

Table 2: Site of involvement of lesion with Histopathological grades of OSCC.

Histopathological Grades of OSCC	Site of lesion					
	Floor of mouth	Gum	Palate	Lip	Tongue	Total
WDSCC	8%(4)	8%(4)	6%(3)	0%(0)	12%(6)	34%(17)
MDSCC	10%(5)	10%(5)	14%(7)	0%(0)	18%(9)	52%(26)
PDSCC	6%(3)	2%(1)	2%(1)	2%(1)	1(2%)	14%(7)
Total	24%(12)	20%(10)	22%(11)	2%(1)	32%(16)	100%(50)

Table 3: Vitamin E levels among study participants

Groups	Mean	SD	p-value (One Sample t-test)
A	0.37	0.20	0.9
B	0.31	0.09	0.0051
C	0.32	0.10	0.05
D	0.35	0.09	0.54
Male	0.311	0.34	0.29
Female	0.13	0.16	0.36
Snff users(A&C)	0.34	0.15	0.42
Non Snuff users(B&D)	0.33	0.10	0.06
Cases (A&B)	0.29	0.15	0.0006
Controls (C&D)	0.35	0.09	0.62
WDSCC	0.37	0.19	0.84
MDSCC	0.33	0.11	0.16
PDSCC	0.29	0.16	0.24

Table 4: Serum Vitamin E levels among cases and controls; snuff users and non-snuff users.

Subjects	Mean	SD	p-value (Student's t-test)
Cases (n=50)	0.33	0.15	0.09
Controls (n=50)	0.34	0.09	
Exposed to snuff (n=50)	0.34	0.15	0.53
Not exposed to snuff (n=50)	0.32	0.09	

carcinoma) (52%), followed by WDSCC (well differentiated squamous cell carcinoma) (34%) and PDSCC (poorly differentiated squamous cell carcinoma) (14%).

Statistically, a significant decline was observed in the mean serum vitamin E levels of cases without a history of snuff use; group B (p-value=0.0051) and controls with a history of snuff use; group C (p-value=0.05).

Mean serum vitamin E levels were significantly lowered (p-value=0.0006; single sample t-test) in OSCC cases. The statistical comparison made of the serum vitamin E status of cases and controls, using

chi-square test, revealed nonsignificant difference (p-value= 0.838).

The nonsignificant difference was observed in the mean serum vitamin E levels in different grades of the tumor.

Statistically negative (OR=0.85) and nonsignificant association (p-value=0.838) was observed between oral squamous cell carcinoma and low serum vitamin E status. Statistically negative (OR=0.31) and nonsignificant association (p-value=0.08) was observed between oral squamous cell carcinoma and low serum vitamin E status among carcinoma patients with the history of snuff use (group A). Statistically positive (OR=2.37) but nonsignificant association (p-value=0.24) was observed between low serum vitamin E status and snuff usage among healthy controls (group C).

## DISCUSSION

Tobacco is the most important established risk factor for OSCC.<sup>10,11,12,25</sup> but there are a large number of studies in which patients who developed OSCC without any history of tobacco.<sup>1,13,17,19</sup>

One of the predisposing factors may be a partial or complete loss of known protective effect of serum vitamin E against the development of the malignancy. The present study is designed to determine and assess the possible role of vitamin E in the development of oral squamous cell carcinoma in such cases. This cross-sectional study includes estimation of serum vitamin E levels in oral squamous cell carcinoma cases and healthy controls with or without snuff usage habit.

This age and the sex-matched study revealed the mean age of 52 yrs a male to female ratio 4:1. However comparable studies give the result of 2:1. Regarding the most frequent site of distribution of OSCC (Tongue) and histological grade of squamous cell carcinoma( moderately differentiated squamous cell carcinoma), our study revealed results which are

consistent with other nationally and internationally published studies.<sup>1,4,5,6,7</sup>

Our study observed the statistically nonsignificant relationship between serum vitamin E status and gender ( $P=0.317$ ). Same observations were made by Aravindh and Lawal.<sup>43,44</sup>

The present study noted a significant decline in mean serum vitamin E levels in oral squamous cell carcinoma cases (Table 3). This is consistent with studies reported by Premkumar and Manoharran.<sup>45,46</sup>

Our study revealed statistically insignificant difference between serum vitamin E status ( $p=0.838$ ) and levels ( $p=0.09$ ) of squamous cell carcinoma cases with respect to healthy controls; contrary to an Indian and Nigerian study which observed statistically significant decline in serum vitamin E levels of OSCC patients with respect to healthy controls.<sup>44,47</sup>

The present study observed statistically negative ( $OR=0.85$ ) and nonsignificant ( $p=0.838$ ) association between oral squamous cell carcinoma and low serum vitamin E status contrary to studies by Raghuwanshi and Lawal which observed positive and statistically significant association.<sup>44,47</sup>

The present study has shown a negative ( $0.31OR$ ) and statistically nonsignificant ( $p=0.08$ ) statistical association of low serum vitamin E and snuff usage with the development of oral squamous cell carcinoma. This observation is inconsistent with a Nigerian study which observed a significant association between oral squamous cell carcinoma and low serum vitamin E status with the history of tobacco use.<sup>44</sup>

This study also showed a substantial decline in the mean serum vitamin E levels among healthy controls with the habit of snuff usage (group C) (Table 3) and positive ( $OR=2.37$ ) but non significant ( $0.24$ ) association between low serum vitamin E status and snuff usage among healthy controls indicating that there exists a relationship between snuff usage and deficient vitamin E status among healthy controls.

A significant decline in mean serum vitamin E levels among oral squamous cell carcinoma patients has been found but could not establish a positive and statistically significant association between oral squamous cell carcinoma and low serum vitamin E status.

The inconsistent results of our study in comparison with others may be due to the small sample size,

sampling technique (nonprobability). Or may be due to different methodology (spectrophotometry instead of Enzyme-linked immunosorbent assay (ELISA) or High-performance liquid chromatography (HPLC) used and strict inclusion and exclusion criteria among carcinoma cases with the specific inclusion of snuff users and non users and exclusion of patients with the history of other etiological factors like smoking and alcohol consumption .

## CONCLUSION

The present cross-sectional study did not establish a positive association between oral squamous cell carcinoma and low serum vitamin E levels or deficient vitamin E status among oral squamous cell carcinoma patients exposed to snuff and not exposed to snuff.

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