ESTIMATION OF SERUM VITAMIN C LEVELS IN ORAL SQUAMOUS CELL CARCINOMA PATIENTS

Muhammad Nisar[1], Muhammad Younas[2], Abdus saboor[3], Sana Ahmad[4], Abbas Saleem Khan[5], Muhammad Mumtaz Khan[6]

[1]Department of Oral Pathology Peshawar Dental College, KP Pakistan

[2]Department of Oral Medicine Peshawar Dental College, KP Pakistan

[3]Dental Surgeon Saidu Group of Medical Colleges Swat, KP Pakistan

[4]Bacha Khan Dental College Mardan, KP Pakistan

[5]Department of Oral Pathology Peshawar Dental College, KP Pakistan

[6]Department of Pathology Peshawar Medical College, KP Pakistan

ABSTRACT

Objective: *To estimate serum vitamin C levels in oral squamous cell carcinoma (OSCC) patients.*

Materials and Methods: *This case control study was conducted at Peshawar Medical and Dental College, and Institute of Radiology and Nuclear Medicine(IRNUM)Peshawar. This study was carried out from March 2017 to February 2018. A total of 60 subjects fulfilling inclusion and exclusion criteria 30 histologically confirmed cases of oral squamous cell carcinoma and 30 Age matched adult individuals of both gender matched reporting to the out patient department of Peshawar Dental College for routine dental checkup or minor dental procedures were used as healthy controls by non-probability consecutive sampling technique. Blood samples were collected from all 60 subjects. Estimation of Serum vitamin C levels was carried out by using ELISA method.*

Results: *The mean age of participants in our study was 42.15±17.24. Oral squamous cell carcinoma was observed normal 30(100%) by comparing with healthy control 29(96.7%) having p value (3.13).*

Conclusion: *Our study concluded with no association between serum vitamin C levels in OSCC patients and healthy controls. Further research with a larger sample size is needed to validate our findings.*

Keywords: *Oral Squamous Cell Carcinoma, Vitamin C, Antioxidants*

INTRODUCTION

Oral cancer occurs in the lips, hard palate, upper and lower alveolar ridges, anterior two thirds of the tongue, sublingual area, buccal mucosa, retro- molar area and floor of the mouth.[1] The most common form of oral malignancy is OSCC.[2] In 2018 it was calculated that around 354,864 people had oral cancer out of which 177,384 were expected to die.[3,4] In global ranking order these cancers are at sixth position of all malignancies.[2,4] There is a wide geographic difference in the occurrence of the oral carcinoma. The occurrence rate changes globally as well as regionally. It represents under 5% of all malignancies in United States, Western Europe and Australia.[5-7] The nations of South Asian Zone which includes Srilanka, Pakistan, Afghanistan, India, Bangladesh, Nepal, Maldives and Iran are especially affected with oral cancer having different prevalence rates.[8,9]

In Pakistan oral cancer is most frequently seen in men with a male to female ratio 2:1.In women it is recognized as the second most common malignancy after the breast cancer.[10] According to age standardized rate of 12.0/100000, Pakistan is ranked among

Correspondence:
Muhammad Nisar
lecturer, Oral Pathology, Peshawar Dental College, Warsak Road Peshawar, Khyber Pakhtunkhwa, Pakistan
Email: nisar756@yahoo.com
Contact: +923005924419

the top 25 countries with respect to frequency of oral cancer. In Pakistan roughly 16000 new cases of oral cancer are diagnosed each year and around 5500 deaths occur due to oral malignancy.[4,11]

The etiology of oral cancer is multifactorial. Various risk factors are responsible for its causation. The most common etiological elements are use of tobacco (both smoked and smokeless), alcohol, betel quid, infection by human papilloma virus and nutritional deficiencies. These factors play a major role in the causation of oral cancer.[2,12-20]

Chewing betel quid with tobacco exposes the oral mucosa to carcinogens including tobacco-specific nitrosamines (TSNA) and nitrosamines from areca nut alkaloids. Moreover reactive oxygen species (ROS) involved in multistage initiation of cancer are additionally produced in significant amount in the oral cavity during mastication.[21,22]

It has been advised by the working group of International Agency for Research on Cancer (IARC) that low intake of fruits and vegetables leads to the development of malignancy.[23,24] Fruits and vegetables (high in vitamin A and C) are depicted as having a defensive role in oral malignancy, while meat and red chili powder are believed to have harmful effects.[25,26]

Extensive work has demonstrated that specific micronutrients diminish the progression of oral malignancy. They include vitamin A, vitamin C, vitamin E, β-carotene, potassium and selenium.[38-43] Antioxidants (β-carotene, retinol, retinoids, vitamin C and vitamin E) are basic elements in diminishing free radical reactions that can cause DNA damage, changes in enzymatic action and lipid peroxidation of cell membrane.[23,27]

Vitamin C (ascorbic acid) is an antioxidant which reduces nitrosation by the formation of nitrosamines, that act as a chemo-preventive agent. The activity of leukocytes and macrophages are also affected by it. Ascorbic acid is additionally engaged with the movement of cytochrome P450 which is critical in the inactivation of strong cancer-causing agents and pro carcinogens. Therefore a lesser intake of vegetables and fruits which are typically abundant in vitamin C may potentiate the development of oral malignancy.[28,29]

The ascorbate disrupts reactive oxygen species(ROS) and changes them into an inefficient semi-hydroascorbate radical. In this way ascorbate effectively reduces in vivo injury to proto-oncogenes consequently decreasing the risk of carcinoma by decreasing the oxidative pressure prompted by reactive free radicals[30,31] The present study is designed to assess the vitamin C levels in OSCC and healthy controls in our region.

MATERIALS AND METHODS

After the approval from the ethical committee of Peshawar Medical College this study was conducted at Peshawar Medical and Dental Collage, Khyber College of Dentistry Peshawar, and Institute of Radiology and Nuclear Medicine Peshawar. A total of 60 individuals after fulfilling inclusion and exclusion criteria were included in this case control study. The study groups comprised of 30 histologically confirmed cases of OSCC and 30 Age matched adult individuals of both gender matched reporting to the OPD of Peshawar Dental College for routine dental checkup or minor dental procedures were used as a controls. Serum vitamin C levels were calculated after taking 5ml Of venous blood from the study participants in yellow top vaccutainers after overnight fast under aseptic conditions. The vaccutainers containing blood was centrifuged at 3000 rpm for five minutes after that serum was isolated in Eppendorf tubes and stored at -20oC till analyzed. Analysis of serum vitamin C levels was done by utilizing Vitamin C ELISA Kit and automatic ELISA micro plate reader .The reference value of E-EL-0011 of Human serum is 6.28-27.16μg/ml . The data was analyzed by using SPSS version 21. Pearson chi- square test was applied to compare the serum vitamin C status between the two groups. P value <0.05 was taken as significant.

RESULTS

The present study included 60 participants out of which 30 were healthy controls and 30 were OSCC. The mean age of the healthy controls calculated was 33.34±13.20 and the mean age of oral Squamous cell carcinoma patients calculated was 49.87±17.52 as shown in the Table 01.

Statistically significant comparison was found among different age groups between serum Vitamin C levels of OSCC patients and healthy controls the P value was less than 0.003 shown in the Table 02.

Among 30 healthy controls 20 (66.67%) were male and 10 (33.33%) were female, while among 30 OSCC 17 (56.67%) were male and 13 (43.33%) were female, statistically insignificant relation was found between the gender and the lesion as shown in the Table 3 below.

Statistical comparison was made between the serum vitamin C levels, OSCC and healthy controls revealed statistically insignificant relation the p value was 3.13 given in table 4.

DISCUSSION

In this study the mean age in OSCC patients was 49.87, which is in accordance with studies done by Khaleel M et.al and Mahmood N et.al while the study done by Minhas S et.al the mean age is 52.74 which is higher than our study. Most of the cases (OSCC) of the present study presented in age above 40 years this finding is consistent with the study done by Azhar N et.al, literature revealed that most of the cases (OSCC) in age above 40 is that most people in our population consume more tobacco.[32-35]

Our study observed statistically significant comparison among different age groups between OSCC and healthy controls with a p value of 0.003, same observations were made by Sharma A et.al in 2019 but it is in contrast to the study done by Asif and

Table 1: Mean age with standard deviation of the study participants

Groups	Minimum	Maximum	Mean	SD	Number
Normal controls	15	68	33.34	13.208	30
OSCC	20	90	49.87	17.536	30

Table 2: Statistical comparison of serum Vitamin C levels in different age groups among OSCC patients and healthy controls

Age group(in years)	Oral Squamous Cell Carcinoma	Controls	P value
10 -19	0(0.00%)	05(16.7%)	0.003
20-39	08(26.7%)	16(53.3%)	
40-59	10(33.3%)	06(20.0%)	
60 and above	12(40%)	03(10%)	
Total	30(100%)	30(100%)	

Table 3: Statistical comparison of serum Vitamin C levels in gender among OSCC patients and healthy controls

Gender	Controls	Oral Squamous Cell Carcinoma	Total	P value
Male	20(66.67%)	17(56.67%)	61.67 %	0.426
Female	10(33.33%)	13(43.33%)	38.33 %	
Total	30(100%)	30(100%)	60 (100%)	

Table 4: Statistical comparison between serum vitamin C levels and the lesion.

Vitamin C Status	Oral Squamous Cell Carcinoma	Controls	P value
Deficient	0(0.0%)	01(3.3%)	3.13
Normal	30(100%)	29(96.7%)	
Total	30(100%)	30(100%)	

Kanwal who observed statistically insignificant association with different age groups, this contrast is due to social habit and patients delay is also a reason.[9,36]

In this study male to female ratio in OSCC, 17 (56.67%) male and 13(43.33%) female in our study is 1.3:1 which is consistent with the studies done by Sahaf R et.al in 2017; however a study done by Naseer R et.al showed male to female ratio of 3.7:1 which is comparatively higher than the participants of our study.[37,38]

The relationship of gender to squamous cell carcinoma in our study turned out to be statistically insignificant (p=0.426) which is identical to the study done by Tandon A et. al in 2018. However the study done by Sharma A et.al in 2019 is inconsistent with our study.[36,39]

According to the present study no significant correlation was found between serum vitamin C levels in OSCC patients and healthy controls; however, in contrast the study done by Raghuwarishi U and his colleagues obtained a statistically significant correlation.[40] These differences in results could be due to geographical variation, patient selection and methods of analysis.

CONCLUSIONS

Although our study concluded statistically insignificant association between serum vitamin C levels in OSCC patients and healthy controls but taking it as a pilot study we recommend a future research with a larger sample size for more comprehensive findings.

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