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ORIGINAL ARTICLE

Determination of the role of serum alpha 1 anti-trypsin protein levels in oral squamous cell carcinoma Patients and in healthy snuff users and non-users

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ABSTRACT

Background: Among head & neck cancers, oral squamous cell carcinoma (OSCC) is the most common malignant neoplasm of lip and oral cavity. The serum analysis of different biomarkers including Alpha 1 antitrypsin, showed various biochemical changes which is considered to be significant in early intervention leading to an increase in survival rate. The objective of our study is to determine serum alpha 1 anti-trypsin (AAT) protein levels in oral squamous cell carcinoma patients and in healthy individuals (tobacco users and non-users) to detect the likelihood of OSCC development.

The rationale of the study is that the early detection of OSCC helps in early intervention which results in an increase in the survival rate.

Methods: This cross-sectional study was conducted from June 2023 to October 2023 at the Chemical Pathology Department of Mercy Teaching Hospital, Peshawar and the Department of Oral Pathology, Peshawar Dental College, Peshawar. A total of 90 subjects were included in the study, divided into 3 groups: 30 subjects having OSCC form Group A. Group B comprised of subjects who were healthy tobacco users. Group C comprised of subjects who were healthy and didn't use tobacco. The evaluation of samples was carried out with alpha1antitrypsin ELISA kit and statistical analysis was performed using SPSS version 19.

Results: A slight rise regarding serum levels was seen for AAT antibody in Group B (healthy individuals tobacco users) (2.96) in comparison to Group C (healthy individuals' tobacco non-users) (2.32). Also, AAT levels were expressed in serum of OSCC patients (2.53).

Conclusion: AAT may be used as a diagnostic marker for OSCC.

Keywords: Alpha 1 Antitrypsin, Oral Squamous Cell Carcinoma, Tobacco

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Introduction

Head and neck cancer accounts 550,000 cases globally and yearly. Among them, squamous cell carcinoma is frequently encountered malignant neoplasm of lip and oral cavity. In Asia cancer incidence is 65.8% with mortality rate 74%. The largest incidence as well as

prevalence with regards to OSCC is found to be in the area known as the Indian subcontinent (1-3).

Regarding Globocan 2020 (Pakistan region), the incidence of oral cavity and lip is 16,959 (9.5%). The number with regards to new cases for females as well as males in all ages for lip and oral cavity is 11,395 (12.9%) and 5564 (6.2%) respectively (4-6).

The risk of development of OSCC is increased due to rampant habits like tobacco chewing, betel quid chewing as well as areca-nut chewing. Approximately, 100 million persons consume tobacco (smokeless) in India and Pakistan. Betel chewing (which includes betel nut and betel quid) is considered common in Pakistan as well as India. These habits are linked to OSCC and HNSCC (7).

Most OSCC cases occur between ages of 50-70 years. However, children as early as 10 years can also be affected (8). According to GLOBOCAN 2020; males are more commonly affected by OSCC (9). OSCC affects tongue in 20-40% cases, followed by floor of mouth, Gingivae, palate, retromolar, labial and buccal mucosa (10).

Despite of therapeutic research, the survival rate is still around 5 years and cases are usually diagnosed in late stages. Further, traditional treatment for OSCC is harmful compromising life of a patient (11).

Serum analysis for early diagnosis and prognosis of cancer is considered helpful as carcinogenesis leads to biochemical variations ultimately increasing level of different biomarkers (12). AAT is considered an important biomarker in oral squamous cell carcinoma. It is comprised of 394 amino acid residues. A significant part or component of the structure is RCL or the reactive center loop. Inhibitory mechanism in regards to AAT is considered remarkable (13). When malignancy occurs, the serum AAT levels will rise considerably in response to the trypsin that is being produced by tumor cells (14).

This study is designed to determine if serum alpha 1 antitrypsin can be utilized for early detection of OSCC for the appropriate management of patient.

Methods

The present cross-sectional study was conducted from June 2023 to October 2023 at the Chemical Pathology Department of Mercy Teaching Hospital, Peshawar and the Department of Oral Pathology of Peshawar Dental College, Peshawar after obtaining approval from ethical review board via letter no Prime/IRB/2023-419). A sample of 90 subjects was taken which was divided into three groups. Group **A**: 30 histopathologically diagnosed patients of oral squamous cell carcinoma with the help of WHO grading system and a history of tobacco use. **Group B**: 30 individuals who did not have OSCC. They were healthy and had a history of tobacco use. **Group C**: 30 individuals who were healthy without the history of OSCC and tobacco use.

Patients who were confirmed histopathologically as OSCC who had not received any kind of treatment and healthy individuals with a history of tobacco for at least 1year were included. Recurrent cases of OSCC and patients with systemic inflammatory conditions were excluded from the study. Written consent was taken from all the subjects. Important data like age, gender, history of tobacco use was entered into a proforma followed by collection of venous blood. The samples were stored in the gel tubes and were centrifuged to get serum placed in special freezers to avoid denaturation at - 20˚C.

To determine the AAT levels in the serum samples ELISA kit was used according to manufactures protocol. The kit was stored at 2–

8 °C prior to use. Venous blood samples were allowed to clot at room temperature for 10–20 minutes and centrifuged at 2000–3000 rpm for 20 minutes, after which the serum supernatant was carefully collected. All reagents and samples were brought to room temperature before the assay. Standards were prepared by reconstituting the supplied standard (19.2 mg/mL) with standard diluent to obtain a 9.6 mg/mL stock solution, followed by serial 1:2 dilutions to generate concentrations of 4.8, 2.4, 1.2, and 0.6 mg/mL, with standard diluent serving as the zero control. Wash buffer was prepared by diluting the 25× concentrate with distilled water. For the assay, 50 µL of standards and 40 µL of serum samples were added to the respective wells; 10 µL of anti-SERPINA1 antibody and 50 µL of streptavidin-HRP were then added, followed by incubation at 37 °C for 60 minutes. After washing the plate five times, 50 µL each of substrate solutions A and B were added and incubated for 10 minutes at 37 °C in the dark. The reaction was terminated by adding 50 µL of stop solution, and optical density was measured at 450 nm using an ELISA microplate reader. The cut-off value of serum AAT levels was 3.670mg/ml (15). Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) ersion 19. Mean and standard deviations were measured for continuous variable e.g. – Age. Chi square test was used to compare categorical variables and t test was applied to compare means. Fisher's exact test was applied where values were less than 5. One way ANOVA was used for comparison between the three groups Probability value of less than or equal to 0.05 (P ≤0.05) was considered statistically significant.

Results

The study was conducted on total of 90 subjects. The study participants consisted of 30 cases of oral squamous cell carcinoma (group A), 30 healthy individuals with history of tobacco use (group B), and 30 participants without history of tobacco usage (group C).

Most of the cases of OSCC (N=10; 33.3%) presented in the age range of 20-40 years.

Among the cases of OSCC 46.7 %(N=14/30) were current tobacco users and 1 case was ex-tobacco user. Most of the cases of OSCC (N=9) were smoked tobacco (ST) users while healthy individuals (Tobacco users) were smokeless tobacco (SLT) users (46.7%; N=14) [Table-1].

Table 1: Composite description (Age, Gender, Tobacco using status) of the study population

Study variables	Group A (OSCC) N=30 (%)	Group B (Healthy Individuals- Tobacco user) N=30 (%)	Group C (Healthy Individuals- Non-Tobacco user) N=30 (%)	Total N=90 (%)	p-value*
AGE					
20-40	10(33.3)	9(30)	8(26.7)	27(30)	0.933
41-50	5(16.7)	7(23.3)	8(26.7)	20(20.2)	
51-60	6(20)	7(23.3)	8(26.7)	21(23.3)	
>60	9(30)	7(23.3)	6(20)	22(24.4)	
Gender					
Male	14(46.7)	28(93.3)	16(53.3)	58(64.44)	0.0002
Female	16(53.3)	2(6.7)	14(46.7)	32(35.55)	
M: F	1:1.14	14:1	!:0.875		
Tobacco using status/habits					
Tobacco user (present)	14(46.7)	21(70)	0(0)	35(38.88)	**
Ex tobacco user	1(3.3)	9(30)	0(0)	10(11.11)	
Non tobacco user	15(50)	0(0)	30(100)	45(50)	

Among the cases of OSCC, the most frequently involved (16.7%; N=5), primary intraoral sites were cheek mucosa, vestibule of mouth and retromolar area (ICD-C06).
Histologically, 56.7% cases of OSCC were diagnosed as, well differentiated squamous cell carcinoma (WDSCC), followed by moderately differentiated squamous cell carcinoma (MDSCC) (30%) and poorly differentiated squamous cell carcinoma (PDSCC) (13.33%) [Table-2].
A statistically non-significant difference was observed for alpha-1 antitrypsin antibody levels among the study participants (Table-3).

Table 2: Clinico-pathological features of OSCC cases

Clinico-pathological Features		Tobacco using status			Statistics	
ICD-10 Code	**Site of development of OSCC lesions**	**Tobacco user N(%)**	**Tobacco non user N(%)**	**Tobacco ex user N(%)**	**Total N(%)**	**p-value***
C00	Malignant neoplasm of Lip Lip (External and inner aspects of lip, commissure of lip)	1(3.3)	0(0)	0(0)	1(3.3)	0.39
C01	Base of tongue (Posterior third, dorsal surface of base of tongue)	1(3.3)	0(0)	0(0)	1(3.3)	
C02	Specified parts of tongue (Borders, anterior-two-thirds, dorsal and ventral surfaces of tongue)	1(3.3)	2(6.7)	0(0)	3(10)	
C03	Gum (Upper and lower gum)	2(6.7)	1(3.3)	1(3.3)	4(16.7)	
C04	Floor of mouth (Anterior and lateral floor of mouth)	0(0)	1(3.3)	0(0)	1(3.3)	
C05	Palate (Hard and soft palate, uvula)	-	-	-	-	
C06	Other and unspecified parts of mouth (Cheek mucosa, vestibule of mouth, retromolar area)	9(30)	11(36.6)	0(0)	20(66.6)	
Histopathological features of OSCC lesion (WHO Grading System)						
WDSCC		8(26.7)	8(26.7)	1(3.3)	17(56.7)	0.100
MDSCC		2(6.7)	7(23.3)	0(0)	9(30)	
PDSCC		4(13.3)	0(0)	0(0)	4(13.3)	
*Pearson's Chi-square test						

Table 3: Comparison of serum AAT levels between OSCC patients, healthy tobacco users, and healthy non-users

Study groups	OSCC cases	Healthy individuals (Tobacco users)	Healthy individuals (Tobacco non- users)	p-value*
Minimum	0.01	0.16	0.30	**0.796**
Maximum	14.68	14.90	14.39	
Mean	2.53	2.96	2.32	
SD	3.425	4.27	3.59	
*One way ANOVA				

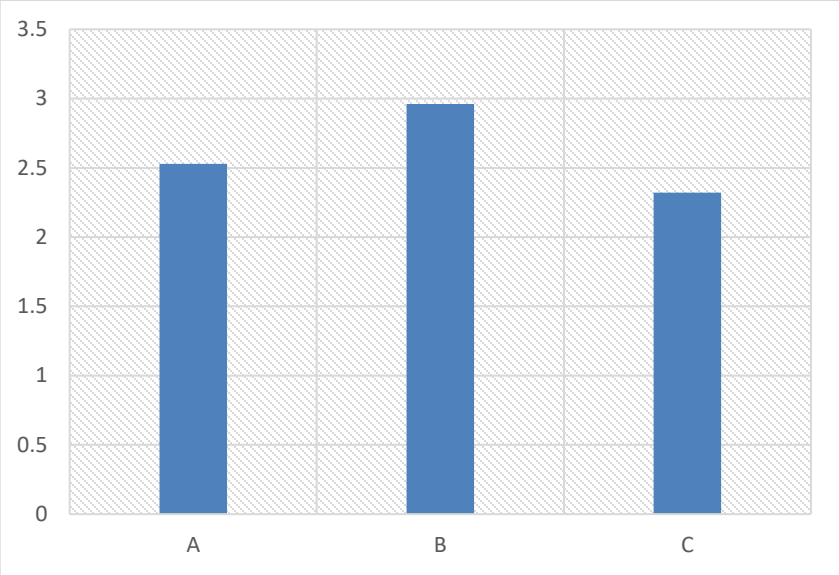

Figure **1: Mean serum AAT levels in groups A, B and C**

Discussion

There are numerous mechanisms involved in invasion and metastasis of cancers. The ability of the malignant cells to produce proteolytic enzymes is believed to be implicated in invasion as it degrades extracellular matrix. It is hypothesized that the increase of AAT with regards to cancer will occur quite possibly as part of a variety of protective mechanisms of host in reaction to the burden of the tumor. Thus, the current study has been undertaken for the estimation and correlation of the serum AAT levels in histologically confirmed cases of OSCC and healthy cases with and without tobacco use.

The mean age of the study participants was 52.63 years. This observation is similar to other studies conducted showing 52.63 and 55.2 years as mean ages (16,17).

In the present study, the gender most affected by OSCC was male (n=58, (64.44%) compared to female n=32, (35.55%). In another study, the male to female ratio with regards to OSCC was 1.3:1(18). Similarly, the findings of another study revealed male as the gender most pretentious by OSCC (n=62, 81.6%) (19). Males are more prone to develop OSCC than females as they are indulged in high-risk behaviors of tobacco and alcohol consumption.

In our study, the sites most frequently involved by OSCC were as follows: cheek mucosa, vestibule of mouth, retromolar area which is comparable to another study in which the most prevalent site for OSCC was buccal mucosa (n:321,31.47%) (20). Some of the studies revealed contrasting findings with tongue and the floor of the mouth as common sites followed by alveolar rim the palate and the buccal mucosa (21). Wolfer et al showed the floor of mouth as main location (22). In another study, the most prevalent site for OSCC was buccal mucosa (n:321,31.47%) (20).

Regarding grades of OSCC based on WHO histopathological grading system, our study revealed that WDSCC (56.7%) was commonly seen followed by MDSCC (30%) and PDSCC (3.3%). A previous study showed comparable findings with WDSCC (58%) as common grade of OSCC while 39% were MDSCC and 2% were PDSCC (22). However, one of the

studies concluded MDSCC as the frequently observed grade. Degree of differentiation demonstrates that MDSCC was the most commonly present (76.5%). Next in line was WDSCC at 18.6% followed by PDSCC at 2.9% (23).

The estimated mean of the serum AAT antibody levels among our groups were as follows: Group A (2.53), Group B (2.96) and Group C (2.32) which revealed a statistically non-significant difference (p=0.796) on comparing AAT values among three groups. These findings are dissimilar with the study which reported a highly significant difference (24). However, a slight rise in serum levels was observed for Alpha-1 antitrypsin antibody in Group B as compared to Group C. Ahuja et al also reported analogous result. There are several studies showing serum analysis of AAT in oral squamous cell carcinoma patients and other malignancies (25). But to our knowledge only one study was reported to determine serum AAT levels in OSCC patients, healthy subjects (tobacco users and non-users) related to our work (24). This divergence in expression might be explained on the basis of different laboratory methods employed, difference in kits used and interpretation of the values.

Limitations

This study has few limitations. Firstly, evaluation of serum AAT with different stages of OSCC was not considered. Secondly, immunohistochemistry was not performed to compare these findings for robust results.

Conclusion

Our study concludes that the commonest age for the OSCC is fifth decade of life with male predominance. Buccal mucosa was the commonest site and WDSCC was the frequently observed grade of OSCC. Serum alpha-1 antitrypsin levels exhibited variation among OSCC patients, tobacco users, and non-users, suggesting a possible role in oral carcinogenesis. AAT may serve as a potential biomarker for OSCC detection; however, larger prospective studies are required to confirm its clinical utility.

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All the authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed.	