Original Article

Comparative evaluation of serum alpha-1antitrypsin levels in patients with oral squamous cell carcinoma and in subjects with tobacco habit without carcinoma

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Abstract

Aim/Objectives: To evaluate serum levels of Alpha-1 antitrypsin in patients with oral squamous cell carcinoma and compare them with that of healthy subjects with and without tobacco habits. **Materials and Method:** The sample of 83 subjects was divided into three groups: 30 subjects with oral squamous cell carcinoma formed Group A. The age, sex, habit matched 23 healthy subjects with tobacco habit formed Group B and 30 healthy without history of consumption of tobacco formed Group C. Analysis of the samples was done using Alpha 1 antitrypsin kit and spectrophotometer. **Results:** There was a 2.33-fold rise in serum levels of Alpha 1 antitrypsin in Group A compared to Group B, 3.71-fold rise in Group A compared to Group C and 1.59-fold rise in Group B compared to Group C ($P < 0.001$). A definite rise in serum Alpha 1 antitrypsin levels in patients with oral squamous cell carcinoma as compared with healthy subjects with and without tobacco habits was observed. **Conclusion:** Alpha1-antitrypsin can be used as an adjunct to various diagnostic procedures implied for the evaluation of oral squamous cell carcinoma.

Keywords: Alpha-1 antitrypsin, oral squamous cell carcinoma, spectrophotometry, tobacco

Introduction

Oral cancer accounts for 30%-40% of all malignancies in India and 2%-4% in western countries.[1] Oral cancer is a major health issue in India, where it ranks among the top three types of cancer in the country.[2] The reason for the high incidence in India is attributed to cultural, ethnic, geographic factors and the popularity of addictive habits such as tobacco. India ranks number one in terms of incidence among men and third among women.[3]

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Cancer is a disorder of cellular behavior. It is known that for a tumor to become invasive and metastasize, the tumor cells must cross the basement membrane. Crossing this membrane requires expression of proteases that can degrade extracellular matrix components and invade surrounding connective tissues, and blood vessels.[4] Theseproteolytic enzymes are released by cancer cells. Two major groups of secretory proteinases, which lead to tumor invasion and metastasis are Matrix serine proteinases (MSPs) and Matrix metalloproteinases (MMPs).[5]

Trypsin is one of the best characterized MSPs[6] which hydrolyzes proteins, including extra cellular matrix proteins. It has been

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reported that trypsin is very potent in activating the latent forms of many MMPs, such as *stromelysin and matrilysins.*[4,5] Hence, the ability of human tumor cell-derived trypsin to activate latent MMPs suggests a role for trypsin in initiating the proteinases cascade that mediates tumor invasion and metastasis.

Several lines of evidence suggest that tumor-derived trypsin contributes to growth and invasion of tumor cells. Human colon carcinoma, fibrosarcoma, esophageal carcinoma and leukemia cell lines have been shown to produce tumor-associated trypsinogen.[7] Since trypsin assessment in routine diagnostic practice is not practical; hence an indirect way to evaluate this serine proteolytic activity is to study its antiprotease in serum.[8] Alpha-1 anti-trypsin (A1-AT) is one such well known, antiprotease of trypsin. It accounts for 90% of the total trypsin inhibitory capacity.[9] As it is an inherent defense mechanism of our body to combat with the proteolysis as much as possible by endogenous secretion of its antiprotease, hence in malignancy, serum A1-AT levels rise markedly against the trypsin produced by the tumor cells.

Numerous studies have reported elevated serum A1-AT in Breast, Gynecological, Liver, Lung, Pancreatic and Head and Neck Cancers, Bronchogenic and Nasopharyngeal Carcinomas.[10-14]

It has been hypothesized that increase in A1-AT in cancer occurs possibly as part of various protective mechanisms of the host in response to the tumor burden. Hence, the present case control study was undertaken to estimate and correlate serum A1-AT levels in histologically confirmed oral squamous cell carcinomas (OSCC).

Aim and Objectives

The aims and objectives of the study include the estimation of serum A1-AT levels in patients with OSCC and in equal number of age- and sex-matched subjects with tobacco habit but without carcinoma.

Materials and Methods

The patients were selected from the outpatient clinic of the Department of Oral Medicine and Radiology in a reputed dental college. All the patients were explained about the entire procedure, and a written informed consent was obtained. Ethical clearance was obtained from the ethical committee of the institution (Date: 15/05/2016).

The study included a total of 83 patients who were divided in three different groups.

Group A consisted of 30 patients with histologically confirmed OSCC irrespective of the site. Group B included 23 age-, sex- and habit-matched individuals with absence of any clinically apparent lesion or cancer. Group C is the control group consisted of 30 age- and sex-matched individuals without tobacco habits and without any clinically apparent lesion.

Patients with any systemic disease, which may alter the serum A1-AT level, medically compromised patients on long-term medications, pregnant women and individuals with any recent history of trauma or surgery were excluded from the study.

A detailed case history was taken and a precise oral examination was performed, followed by collection of 3 ml of venous blood which was subjected for separation of serum. The serum sample obtained was stored at −20°C until further analysis.

Evaluation of A1-AT was done using Spectrophotometry A1-AT kit. The Reagents used were TRIS\PEG buffer pH 7.5, Antiserum Anti Alpha 1 Anti Trypsin and Normal saline for Serum dilution.

Assay conditions for the test were wavelength of 340 nm, temperature of 37°C and Cuvette light path of 1 cm. Spectrophotometer adjustment was done to zero with distilled water. Now Anti serum Anti Alpha 1 Anti Trypsin, that is, Reagent 2 was diluted with buffer solution (Reagent 1) in the ratio of 1: 31. This working reagent is stable for 2 weeks at 2°C- 80°C. The serum samples were diluted with saline solution in the ratio of 1: 31. After this, 50 µl of diluted serum sample was mixed with 1 ml working reagent in a Cuvette. The Cuvette was then placed in spectrophotometer that was initially adjusted to zero and the absorbance A was read after 10 minutes of working reagent addition. The calibrator was diluted serially from 1\10 to 1\80 by making use of normal saline as a diluent. Again with the help of spectrophotometer optical density of calibrator was recorded.

The absorbance for each calibrator was calculated and the values were plotted against the concentration in a calibration curve. A1-AT concentration in the sample was calculated by interpolating its value on the calibration curve. The formula used for the calculation was as follows:-

$$\frac{\text{Optical density of Sample} \times \text{Dilution factor}\ (31)}{\text{Optical density of calibrator}}$$

All the variables computed from the study were statistically analyzed.

Results and Observations

In group A, out of 30 subjects with oral cancer 80% were males and 20% were females with an age range of 28-70 years and the mean age being 47.96 ± 11.18 years.

In the present study, subjects with OSCC involving various sites were included such as Buccal mucosa 36.66%, Gingivo-buccal sulcus 33.33%, Lower alveolus 10%, Palate 6.67%, tongue 3.33%, Retromolartrigone 3.33%, Lip 3.33% and Floor of the mouth 3.33%.

Twenty eight subjects (93.33%) enrolled in the present study in group A were having either habit of tobacco chewing, smoking, alcohol consumption, pan, betel nut and lime chewing or a combination [Table 1]. Table 1 also shows the values of A1-AT in group A. A history of tobacco habits was present in 100% males and 66.6% females.

Table 2 shows the habit distribution and Alpha 1 Antitrypsin levels in Group B and Table 3 shows Antitrypsin levels in Group C.

In Group A, mean serum AI-AT was 1228.8960 mg/dl. In Group B and in Group C, mean serum AI-AT levels were 528.2435 and 331.6133 mg/dl respectively [Table 4]. The mean serum AI-AT level was highest in Group A and lowest in Group C.

To test the homogeneity among the three groups ANOVA (one way analysis of variance) was done which showed that the *P* value between groups is 4.36E-43 which is *highly significant* [Table 4]. Mann Whitney U Test showed Z value between Group A and Group B to be 6.1914 which indicates a highly significant statistical difference ($P < 0.001$) between these two groups. The Z value between Group B and Group C and between Group A and Group C was also found to be statistically highly significant ($P < 0.001$). The *P* value between all the groups was again *highly significant* [Table 5].

The results show significant elevation in serum A1-AT values in patients with carcinoma compared to that of control groups. Similarly, a significant elevation in serum A1-AT values in subjects with tobacco habit as compared with that of subjects without the habit were found.

Discussion

The diverse mechanisms involved in tumor invasion are poorly understood. Production of proteolytic enzymes by malignant tumor cells is believed to be essential for the ability of the tumor to invade and degrade extracellular matrix. An increased concentration of trypsin in cancer is indicative of poor prognosis.

As the estimation of trypsin level is tedious and expensive, thus an alternative is to evaluate the concentration of A1-AT in the serum. Various studies have shown elevated levels of this antiprotease in numerous malignancies.[13-15] Evaluation of this A1-AT can give an estimate of the magnitude of the proteolysis occurring due to malignancy.

Table 1: Age, sex, habit distribution and A1-AT levels in Group A

Age	Sex	Tobacco Chewing	Smoking	Gutka	Alcohol	Pan, betel nut, lime	Level of A1-AT
45	F	++					1090.3
65	F	-	-		-	-	925.2
60	M		+				1346.8
55	F	+++					980.3
70	F	++					1186.5
40	M	++					1346.8
45	M		++		+		1276.0
50	F	+					1154.5
45	M		++				1346.8
70	M	+++				+++	1008.5
58	M	+++				+++	1242.0
60	M	+++					1348.2
33	M			+			1100.5
50	M		++			++	1250.5
34	M			+	+		1346.6
58	M	++					1159.0
55	M	+++	++				1678.2
30	M			++	++		1115.0
28	M	+				+	1212.0
45	M	+++				+++	1263.5
60	M	++					999.8
58	M		+++				1773.5
55	M	+++				+++	1280.0
52	M	+					994.1
52	M			+	++		1290.0
40	M	+++				+++	1275.5
40	F	-	-	-	-	-	965.3
50	M	+++				+++	1237.8
47	M		+++				1148.0
36	M						1526.2

Table 2: Age, sex, habit distribution and A1-AT levels in Group B

Age	Sex	Tobacco Chewing	Smoking	Gutka	Alcohol	Pan, betel nut, lime	Level of A1-AT
45	F	+++					548.2
60	F	+++					508.4
60	F	++					497.3
42	M	++				++	523.4
48	M		++		++		600.0
46	F	+++					559.3
45	M		++				608.4
68	M	+++				+++	589.5
55	M	+++				+++	533.0
60	M	+++				+++	622.3
33	M			+			439.8
33	M			++			489.6
54	M	+++					500.4
30	M	++		++			86.7
25	M	+				+	86.3
45	M	++				++	499.0
60	M	++					445.2
54	M		+++		+		642.2
60	M	+++					505.8
48	M	+					486.4
40	M	++				++	511.0
50	M	++				+++	515.2
50	M		+++		+		552.2

The present study was an attempt to estimate the serum levels of A1-AT sampled from subjects with oral cancer. In Group A, 80% patients were males and 20% were females. Present study reported male preponderance which is in accordance to studies by Dhanuthai *et al.*,[16] Monteiro *et al.*,[17] Falaki *et al.*[18] and Troeltzch *et al.*[19]

The age of the subjects ranged from 28 and 70 years and 70% subjects were above 45 years which shows that the incidence of OSCC is more in elderly subjects. In the study conducted by Nagy KN *et al.*[20] patients mean age was 52.8 ± 8.2 years which is slightly higher than our study. Burkhardt and Reinhard[21] also reported oral cancer as a disease of elderly age. In today's time, there are 4%-13% of the cases of OSCC that affects subjects younger than 45 years of age as stated by Gupta *et al.* 2018.[22] Results of previous studies in literature, shows that the age of initiation of oral cancer is declining progressively due to increased frequency of tobacco consumption from younger age and easier availability of refined forms of tobacco products.

In the present study, the site of occurrence of carcinoma was highest in buccal mucosa followed by gingivo-buccal sulcus. Similar distribution of site was observed by **Soben Peter**,[23] Gupta *et al.* 2018[22] and Khan *et al.*[24] where buccal mucosa was the prime site of occurrence of oral cancer. Chidzonza[25] reported gingiva and tongue as the most common sites for oral cancer. The carcinogens in the oral cavity after mixing with saliva have the tendency to pool at the bottom of the mouth and these sites are covered by thin and non-keratinized mucosa. As a consequence, they provide less protection against the carcinogen.

India has one of the highest incidences of oral cancer in the world.[26] In the present study, 93.33% had the habit of consuming tobacco either in chewing or smoking form. Sanghvi *et al.*[27] reported that consuming tobacco increases the risk for developing oral cancer. In our study, 2 out of 6 females gave no history of tobacco which can be attributed to genetic susceptibility, decreased immune surveillance, malnutrition or viruses.

On comparing the rise of serum values of A1-AT between three groups, there was a **2.33-fold** rise in serum levels in Group A as compared with Group B, **3.71-fold** rise in Group A as compared with Group C and **1.59-fold** rise in Group B as compared with Group C. The differences among serum values between the three groups were highly significant ($P < 0.0001$).

Our results are in accordance with the studies done by Millan[8] and Gregory T. Wolf[28] where levels of serum A1-AT were significantly higher in cancer patients than control groups. In contrast to our findings, Kanemitsu *et al.*[14] found an increase in levels of A1-AT in patients with OSCC but no statistical difference could be determined between the cancer and control group.

The mean serum levels of A1-AT were significantly elevated in Group B as compared to Group C. Petridou in 1993,[29] Wolf *et al.*[28] and Fouad *et al.*[30] also reported significant elevation of A1-AT levels in subjects with habit of smoking as compared to non-smokers. Our study included subjects with tobacco habit in both smoke and smokeless forms and it was found that

serum levels of A1-AT were raised considerably irrespective of the type of habit. This rise in serum levels of A1-AT in tobacco users may be related to the immunosuppressive effect of tobacco products.

No statistical difference in A1-AT serum levels was found between males and females in both the control groups. Millan[8] and Petridou[29] also observed no difference in A1-AT levels based on sex. However, present study observedthe mean levels of A1-AT lower in females in cancer groupas compared to males. This might be attributed to the fact that 2 out of 6 females included did not have any tobacco habit which is known to cause an elevation of serum A1-AT levels. Although, there was an increase in serum A1-AT level in these 2 female subjects but the levels were comparatively lower as compared to other cancer subjects.

Table 3: Age, sex distribution and A1-AT levels in Group C

Age	Sex	A1-AT levels
45	F	291.8
65	F	384.2
60	M	360.5
55	F	359.0
70	F	384.6
40	M	422.0
45	M	358.4
50	F	359.5
44	M	292.0
68	M	211.8
58	M	273.0
60	M	248.4
34	M	244.6
50	M	378.0
35	M	325.5
59	M	344.0
55	M	286.5
29	M	284.0
30	M	300.3
45	M	314.6
58	M	333.4
59	M	378.0
54	M	367.3
52	M	311.2
51	M	348.0
40	M	357.0
40	F	292.9
50	M	347.7
48	M	389.4
36	M	400.8

Table 4: Comparative mean values of A1-AT in all the three groups

S. No	Group A	Group B	Group C
Mean	1228.8960	528.2435	331.6133
S.D	196.2316	54.1641	50.7936
Min.	925.2	439.8	211.8
Max.	1773.5	642.2	422.0

The association of increased levels of A1-AT with various carcinomas has been studied by many authors, namely, Wolf *et al.*,[28] Lamoureux *et al.*,[11] Vasishtha *et al.*[10] and Chawla *et al.*[12] **All of them found that the levels of A1-AT were significantly raised with the increasing tumor burden thus serving as a prognostic marker for the disease process.**

Various authors have attributed different reasons for the increase in A1-AT level in cancer patients. According to some authors, the level of anti-proteases is inversely proportional to parameters of cellular immunity. Cancer patients often demonstrate an immunocompromised status at the time of diagnosis; hence, there is an increase of A1-AT levels in such patients.

As a protective mechanism, our body tries to limit the proteolysis by secretion of antiproteases which renders proteases incapable of their function. The invasion, angiogenesis or cellular signal pathway of cancer cells can be blocked by directing specific inhibitors of proteolytic enzymes against them; generating a cytostatic rather than a cytotoxic effect [Figure 1]. This cytostatic therapy in form of antiproteases can constitute an alternative therapy that can complement conventional cytotoxic therapy. It can also be very useful in cases where surgery is contraindicated or in inaccessible sites to improve patient's quality of life.

Conclusion

We can conclude that there is a definite rise in serum A1-AT levels in OSCC. The rise can possibly be as part of various protective mechanisms of the host in response to the tumor burden, or may also be related to a normal immunoregulatory mechanism. Hence, A1-AT can be used as an adjunct to various diagnostic procedures implied for the evaluation of OSCC.

The limitation of the present study was that while evaluating serum values of A1-AT, the staging of oral cancer was not

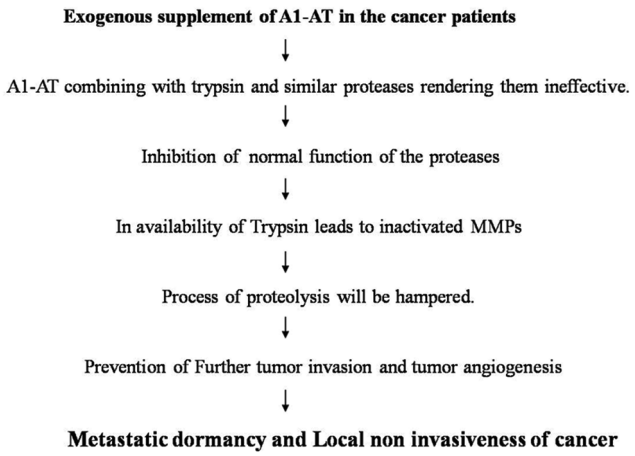

Figure 1: Role of A1-AT in obtaining metastatic dormancy

Table 5: One-way analysis of variance (ANOVA)

Source of Variation	Sum of squares	Degree of freedom	Mean of squares	Variance value	*P*
Between Groups	13132686.16	2	6566343	418.2182	4.36E-43
Within Groups	1256060.697	80	15700.76		
Total	14388746.85	82			

Probability (*P*) value for the three groups

	Groups A and B	Groups B and C	Groups A and C
P	3.20295 E-22	2.35425 E-16	5.04497 E-28
	Highly significant	Highly significant	Highly significant

considered, because the patients due to lack of awareness reported when they are already in a later stage of malignancy. Therefore, further studies with larger sample size need to be carried out in oral cancer in different stages so as to assess the prognostic significance of A1-AT in OSCC.

Future Prospective

Very few studies have been done to evaluate A1-AT in oral cancer. Further clinical trials and extensive qualitative studies on antiproteases are needed to assess their utility for therapeutic purposes. Exogenous antiproteases if given can combat with the excess protease which will help to prevent cancer invasion. Antiproteases can emerge as a new paradigm of treatment modality against oral cancer invasion.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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