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Evaluation of secretor status by the presence of ABO (H) blood type antigens from saliva in patients with oral potentially malignant disorders and oral cancer

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Abstract

Context: The blood group ABO antigens are cell surface carbohydrates distributed abundantly in epithelial tissues. ABO (H) blood group antigens are being researched aggressively as many evidences are pointing to their role in the cellular onco-conversion.

Aim: To assess the secret or status of ABO (H) antigens in patients with oral potentially malignant disorders (OPMD), oral cancer (OC) and healthy controls; and to analyse the association between the same.

Settings and Design: This was a cross-sectional study conducted in a tertiary care teaching hospital in Andhra Pradesh, India.

Methods and Material: 180 participants were recruited with 60 patients each with OPMD (Group II) and OC (Group III), respectively, and 60 healthy controls (Group I). Saliva was tested for presence of ABO(H) antigens. Secretor status of the patients in the three groups was analysed along with their association with various pre-malignancies and malignancy.

Statistical analysis used: The association of secretor status and the three groups were analyzed using Chi-square test.

Results: In healthy controls, the percentage (70%) of secretors was more compared to OPMD group (33.3%) and OC group (23.3%). Of all 3 groups, non- secretors were more in OC group (76.7%) compared to healthy controls (30%) & OPMD group (66.7%). The comparison of secretor status between all three groups was statistically significant.

Conclusion: The study observed that non-secretors were found to be associated more with OC and OPMD. This

finding infers that absence of blood type antigens in saliva can act as risk factor for OPMDs and oral cancer.

Keywords: Leukoplakia, premalignant, body fluid, blood grouping, agglutination.

Key messages

- A significant association exists between salivary secretor status of blood type antigens and occurrence of OPMD/OC.
- Absence of blood type antigens in saliva can be a possible risk factor for OPMD and OC.
- The blood group most frequently associated with oral cancer was group O; with OPMD was group B; and least associated with OC and OPMD was AB group.

Introduction

The term 'cancer' was derived from the Latin word 'karkinos' meaning crab, because they adhere to any part that they seize upon in an obstinate manner, similar to a crab.[1,2] Oral cancer is a serious and growing problem in many parts of the globe.[3] It is the sixth most common cancer in the world.[4] Tobacco and alcohol consumption are regarded as the primary risk factors for oral cancer.[5]

The annual estimated incidence is around 2,75,000 for oral and 1,30,300 for pharyngeal cancers excluding nasopharynx; two-thirds of these cases occurring in developing countries.[6]

In 2005, WHO recommended the term "potentially malignant disorders" to refer to entail what were earlier referred to as precancer or premalignant lesions and conditions. It includes leukoplakia, erythroplakia, lichen planus, submucous fibrosis, erythroleukoplakia, palatal lesions in reverse smokers, discoid lupus erythematosus, syphilis, sideropenic dysphagia and actinic keratosis.[7]

The blood group ABO antigens are cell surface carbohydrates distributed abundantly in epithelial tissues. ABO (H) blood group antigens are being researched aggressively as many evidences are pointing to their role for the cellular onco-conversion.[8]

People who possess the ability to secrete blood group substances (ABO) in the saliva are referred to as 'secretors' whereas others who lack such ability are referred to as 'non-secretors'. The term 'ABH secretor', refers to secretion of ABO blood group antigens in fluids such as saliva, sweat, tears, semen, and serum. Secretion of antigens will be in accordance with one's blood group.[9] Altered blood group antigens in malignant oral tissues may indicate increased cell migration and show lack of expression of A/B antigens. [10]

A study by Bakhtiari S et al., among OPMD patients inferred that the inability to secrete blood group antigens in saliva could be regarded as a host risk factor.[11] Another study conducted by Jamil S et al., which evaluated the secret or status in fixed tissue section suggested that this method be used for surveillance of premalignant lesions in at-risk individuals who are non-secretors.[12] A study by Chordia TD et al., did a similar study on Croatian-European population which could not confirm the hypothesis that secretor status had an influence on development of oral cancer.[13] While, a study from Argentina done by Cerovi R et al., concluded that non-secretor status was risk marker for OC. [14] The relationship between secretor status, OPMD and OC is debatable and further studies are required to comprehend the correct association.

This study aimed to evaluate the secretor status of ABO (H) blood type antigens from saliva in patients with potentially malignant disorders and oral cancer, and compare it with that in healthy controls. The study also attempted to assess any possible association between the same.

Subjects and Methods

Source of data: This was a cross-sectional study conducted in the department of Oral Medicine and Radiology in a tertiary care teaching hospital in Andhra Pradesh, India. The study was approved by the Institutional Ethical Committee.

180 subjects were included in the study and were divided into three groups:

Group 1- 60 normal individuals as control group

Group 2- 60 patients with oral potentially malignant disorders

Group 3- 60 patients with oral cancer

Inclusion criteria

Patients diagnosed clinically and histopathologically with potentially malignant disorders & oral cancer was included in the study.

Clinically healthy individuals without any co-morbidities, age and sex matched with the cases, were included in the control group.

Exclusion criteria

Subjects who were undergoing treatment (pharmacotherapy, chemotherapy and/or radiotherapy) for oral potentially malignant disease and oral cancer were excluded from the study.

All the subjects enrolled were explained about the purpose of current study in the local language. A written informed consent was obtained from each subject before starting with the procedure.

2-3ml of non-stimulated saliva was collected from each patient, by asking them to spit in a sterile container for 5 minutes.[15] Also, 2 to 3 ml of blood was collected from each subject by venipuncture under sterile conditions to prepare red blood cell suspension. The blood sample was mixed with normal saline and centrifuged, to obtain red blood cell suspension.

The samples were tested for ABO blood grouping by hem agglutination slide method.[16]

Establishing the secret or status in the saliva: Saliva was collected using spitting method. [15] The saliva sample was tested for presence of ABO (H) antigens immediately after collection using Anti A & B antiserum by agglutination inhibition test. 2-3ml of non-stimulated saliva were collected from each patient, by asking them to spit in a sterile container for 5 minutes, and then the saliva was poured into a sterile test-tube shut with a rubber or a plastic cover. Test-tube was left to rest for approximately 10 minutes in a boiling water bath (to destroy enzymes). After that, supernatant was extracted by centrifugal force of 1700 turns through 10 minutes.

By applying the Wiener agglutination test, the secret or status was analyzed.[17] Commercial serum (Anti-A and Anti-B) used in this experiment, was diluted in a salted physiological solution (saline) in proportion 1:10, the same proportion that the saliva was diluted in. It is necessary, to dilute the commercial antiserum so that its antibody titer more closely matches the antigen level in the saliva.

The following antiserum is then placed into test-tubes marked I to IV

- 1 drop of saliva + 1 drop of anti-B serum.
- 1 drop of saliva + 1 drop of anti-A serum.
- 1 drop of physiological solution + 1 drop of anti-B serum.
- 1 drop of physiological solution + 1 drop of anti-A serum.

After 10 minutes at room temperature, 1 drop of 2–3% of suspension ‘A’ erythrocytes were added into sterile tube II and IV, and 1 drop of suspension ‘B’ erythrocytes into tube I and III. All the test tubes were agitated, and left at room temperature. After one hour the results were ready for reading.

Agglutination in tube I was a result of the presence of secretors in saliva, i.e. of secretor A, while the agglutination in tube II was a proof of B secretor. The absence of agglutination in tubes I and II indicated AB secretor, and at the same time agglutination in tubes I and II proved that the person was non-secretor. Test-tubes III and IV were controls and agglutination occurred in both test tubes.

All the Statistical analysis was done using software SPSS (Statistical Package for the Social Sciences) version 20.0. A p-value of <0.05 was considered as statistically significant. Comparison of mean values among the groups was done using Chi-square test.

Results

A total of 180 patients of both genders were evaluated based on their secretor status. Age distribution of patients in Groups I, II and III expressed in mean and standard deviation (SD) have been shown in [Table 1] and [Graph 1]. Gender distribution of patients in control group and the study groups showed a male predominance [Table 2] [Graph 2]. The association between the three groups and secretor status has been tabulated in [Table 3] [Graph 3], which showed a higher prevalence of secretors and non-secretors in control group and Group III, respectively.

The Comparison of secretor status between all three groups; secretor status between Group I and Group II, Group I and Group III; and secretor status within the subgroups of group II showed statistically significant associations. [Table 4, 5, 6] The comparison of secretor status between Group I and Group III showed a high statistical significance (P< 0.001). The relation between blood groups and secretor status statistically significant association in Group I and Group III. [Table 7].

The association of secretor status between Group II and Group III was not statistically significant (P< 0.390). [Table 8]

Discussion

In the present study, the age of the affected patients with PMDs ranged between 25- 58 years, with a mean age of 36.13 yrs and 33.3% (20) of the patients were aged less than 30yrs. This was in accordance with the findings given by Misra et al. who found that the incidence of premalignant lesions of the oral cavity showed a predilection for younger age groups, possibly due to the increase in intake of pan masala and other related intoxicants.[18] In our study most of the patients in OC group belonged to the older age group. This finding was in agreement with the studies done by Pulino BFB et al and R Shenoy et al, who reported oral malignancies to be predominant in the 5th and 6th decades of life. [19, 20]

According to Antony George et al, PMDs are predominantly seen in males compared to females due to the increased habitual use of tobacco and alcohol.[2] The study also reported that leukoplakia and OSMF were predominately seen in males compared to females. In present study, OPMD group included 44 male patients and 16 female patients. Also, patients affected with leukoplakia (group IIA) were all males.

Majority of OPMD patients were non-secretors in the present study, whereas in control group most of the patients were secretors. This was in accordance with the study done by Rai P et al who reported that 87% of patients with OPMD were non secretors.[21]

A study by Bakhtiari S et al., reported 84.4% of the OLP patients to be secretors. In the present study, considering the OLP patients in the OPMD group, 60.0% were secretors and the rest 40% were non-secretors. Bakhtiari S et al., however, concluded that study did not indicate a significant difference in salivary secretor status between OLP patients compared with controls. This could be due to the small number of OLP sample included in the study.[11]

In the present study a significant difference was found among the OPMD sub groups. In group II C, all OSMF patients were non- secretors. These findings were similar to a study done by Kaveri Hallikeri et al. The study suggested that there is a correlation between salivary secretor status and the development of OSF, with non-secretors being at a greater risk.[22]

The assessment of the comparison of secretor status between OPMD and OC groups showed statistically non-significant value, in our study. Non secretors were more in OC group compared to OPMD group. This implies that the secret or status is associated with increased risk for incidence of oral cancer. Findings of studies by Carlos Campi et al., Campi C et al., and Alejandra Moreno et al., were in agreement with that of present study as they observed higher number of non-secretors in their oral cancer groups [23,24,25]

Similarly, the comparison of secretor status between control group and OC group showed statistically significant association in the current study. 70% of individuals in control group were secretors whereas only 23.3% of patients in OC group were positive for secretor status. These findings were consistent with the results of studies by Jamil S et al., and Cerovic R et al.,. [12,14]

In OPMD group, blood group O followed by A and B groups were non-secretors. Similarly, in OC group most of the non-secretors belonged to blood group O followed by groups A, B & AB.

Interestingly enough, the present study revealed that patients with blood group B are seen to be affected more with potentially malignant disorders compared with other blood groups. The frequency of occurrence was in the sequence of B, A, O and AB blood group. This was in agreement with a study done by Jyothi et al. [26]

Within the subgroup of group II, most oral lichen planus patients belonged to blood group B. This finding was in consistent with the study done by Bakhitiari et al., in which the frequency of distribution of blood groups of OLP patients was blood group B followed by O, A and AB. [11]

In our study, most of the oral submucous fibrosis patients were blood group A. This finding was similar to the studies by Chordiya et al & Ramesh et al. Chordia et al., who concluded that people with blood group A are 3.98 times at a greater risk to develop OSMF. [13,27]

In addition, the present study observed that the frequency of blood groups associated with oral cancer was in the sequence of O, B, A and AB. The highest incidence of oral cancer was seen in blood group O compared to other blood groups. However, other similar studies in literature by Akhtar et al and Mortazavi et al found that blood group of B was more predominant in OC patients. [28,29] Akhtar et al study was done on North Indian population and Mortazavi et al studied Iranian population, whereas the present study assessed South Indian population. The variation in the results could be possibly due to the differences in the study population characteristics.

Most of the studies from literature showed agreement with the observations of the present study, thus indicating that the patient's salivary secretor status plays an important role in predicting the risk potential for PMDs and oral cancer.

Conclusion

Assessment of secretor status revealed that the percentage of non-secretors was the highest in OC group followed by OPMD group, when compared to healthy controls. This suggests that non-secretor status of patients might act as a possible risk factor for susceptibility to oral cancer and potentially malignant disorders. However, the difference was statistically insignificant. This might be because of smaller sample size considered for the present study.

Controversies raised by these studies & Future recommendations for research

The study does not raise any controversies. However, it has few limitations. This was a single centre study with a small sample size. Larger multi-centre studies on individuals of different ethnicities are required to establish the association between secretor status, OPMD and OC.

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Legend Tables

Table 1: Age distribution in Group I, Group II and Group III

Groups	Min	Max	Mean
GROUP I	24.00	73.00	48.53
GROUP II	25.00	58.00	36.13
GROUP III	25.00	80.00	52.33

* Age distribution of patients in group I was within a range of 24 – 73 yrs with a mean value of 48.53 (SD 15.80). Age distribution in group II was within a range of 25- 58yrs with the mean value of 36.13 (SD 9.47). Age distribution in group III was with a range of 25- 80yrs with the mean value of 52.33 (SD 13.30).

Table 2: Gender distribution in Group I, Group II and Group III.

GENDER	GROUP I	GROUP II	GROUP III
	n(%)	n(%)	n(%)
MALE	34 (56.7)	44 (73.3)	32 (53.3)
FEMALE	26 (43.3)	16 (26.7)	28 (46.7)
Total	60 (100.0)	60 (100.0)	60 (100.0)

*All the three groups showed a higher proportion of men compared to females

Table 3: Relation between Groups and Secretor Status.

GROUPS	Secretor	Non secretor	TOTAL	Chi-square value	P value
	n (%)	n (%)	n (%)	14.848	0.001
GROUP I	84 (70.0)	36 (30.0)	60 (100.0)		
GROUP II	20 (33.3)	40 (66.7)	60 (100.0)		
GROUP III	14 (23.3)	46 (76.7)	60 (100.0)		

*The percentage (70%) of secretors were more in healthy control group (Group I) when compared to group II and group III. of all 3 groups, non- secretors were more in oral cancer group (group III).

Table 4: Comparison of Secretor Status between Group I and Group II

GROUPS	Secretor	Non secretor	TOTAL	Chi-square value	P value
	n (%)	n (%)	n (%)		
GROUP I	42 (70.0)	18 (30.0)	60 (100.0)	8.076	0.004 Significant
GROUP II	20 (33.3)	40 (66.7)	60 (100.0)		

Statistical Analysis: Chi-square test. Statistically significant if P<0.05

Table 5: Comparison of Secretor Status between Group I and Group III.

GROUPS	Secretor	Non secretor	TOTAL	Chi-square value	P value
	n (%)	n (%)	n (%)		
GROUP I	42 (70.0)	18 (30.0)	60 (100.0)	13.125	0.001 Significant
GROUP III	14 (23.3)	46 (76.7)	60 (100.0)		

Statistical Analysis: Chi-square test. Statistically significant if P<0.05

Table 6: Secretor status in group II subgroups

Diagnosis	GROUP II			Chi-square value	P VALUE
	Secretor n (%)	Non secretor n (%)	Total n (%)		
Leukoplakia	8 (40.0)	12 (30.0)	20(33.3)	8.400	0.015 Significant
Oral lichen planus	12 (60.0)	8 (20.0)	20 (33.3)		
Oral submucous fibrosis	0 (0.0)	20 (100.0)	20(33.3)		
Total	20 (100.0)	40 (100.0)	60 (100.0)		

Prevalence of Oral lichen planus was more in secretors whereas OSMF was more prevalent in non-secretors.

Statistical Analysis: Chi-square test. Statistically significant if P<0.05

Table 7: Relation of Blood groups and Secretor Status in Group I, Group II and Group III of examinees.

Blood Groups	GROUP I		GROUP II		GROUP III	
	Secretor n (%)	Non secretor n (%)	Secretor n (%)	Non secretor n (%)	Secretor n (%)	Non secretor n (%)
O+	0 (0.0)	18 (100.0)	0 (0.0)	16 (100.0)	0 (0.0)	22 (100.0)
O-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
A+	18 (100.0)	0 (0.0)	8 (44.4)	10 (55.6)	4 (28.6)	10 (71.4)
A-	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	2(100.0)
B+	24(100.0)	0 (0.0)	12 (54.5)	10 (45.5)	10 (55.6)	8(44.4)
B-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AB+	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)	0 (0.0)	4 (100.0)
AB-	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	42 (70.0)	18 (30.0)	20 (33.3)	40 (66.7)	14 (23.3)	46(76.7)
Results	Chi-square value=30.000 P value=0.000; Significant		Chi-square value=7.727 P value=0.102; Not Significant		Chi-square value=9.592 P value=0.048; Significant	

*The frequency of occurrence of blood groups was in the sequence of B, A, O and AB blood group in group II whereas in group III, the frequency of blood group associated with oral cancer was in the sequence of O, B, A and AB Blood group.

Statistical Analysis: Chi-square test. Statistically significant if P<0.05

Table 8: Comparison of Secretor Status between Group II and Group III. Statistical Analysis: Chi-square test. Statistically significant if P<0.05

GROUPS	Secretor	Non secretor	TOTAL	Chi-square value	P value
	n (%)	n (%)	n (%)		
GROUP II	20 (33.3)	40 (66.7)	60 (100.0)	0.739	0.390 Not Significant
GROUP III	14 (23.3)	46 (76.7)	60(100.0)		

Statistical Analysis: Chi-square test. Statistically significant if P<0.05