Acta Biologica 28/2021 | www.wnus.edu.pl/ab | DOI: 10.18276/ab.2021.28-04 | 33–40

ISSN (print): 2450-8330 | ISSN (online): 2353-3013



# Study of ABO blood grouping and secretor status among habituals – A case control study

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Keywords blood group antigens, habits, immunoglobulins, oral submucous fibrosis, saliva

Abstract The study was conducted to analyse ABO blood group and secretor status using saliva among habituals and healthy controls. The relationship between ABO blood group, secretor status of habituals and oral potentially malignant disorders (OPMDs) was assessed. Salivary secretor status was studied in 100 individuals. Clinical and habit history was noted. Group I had 50 age and sex matched healthy controls; group II had 50 individuals having habit history, with or without PMD. With 1ml of unstimulated saliva from each, Wiener agglutination test was performed to know blood group and secretor status. The relationship between ABO blood groups, secretor status, and OPMDs was assessed using Chi-square test and odds ratio. Group I had blood groups A (44%), B (32%), O (14%), AB (10%) and group II had A (32%), B (26%), O (34%), AB (8%). Non-secretor status in group I was 18% and group II, 82%. In Group II, 20% nonsecretors presented with PMD, predominantly oral submucous fibrosis followed by leukoplakia and lichen planus. A definite correlation between salivary secretor status and development of PMD indicates non-secretors possess greater risk of developing oral lesions. Among the habituals, blood groups B and O were at higher risk than A and AB.

Badanie grup krwi ABO i statusu wydzielania wśród osób żujących tytoń – badanie kontrolne przypadku

Słowa kluczowe antygeny grup krwi, nawyki, immunoglobuliny, zwłóknienie podśluzówkowe jamy ustnej, ślina

Streszczenie Badanie przeprowadzono w celu porównania wpływu grupy krwi ABO i statusu wydzielania śliny wśród osób mających nawyk żucia tytoniu i osób zdrowych z grupy kontrolnej. Oceniono związek między grupą krwi ABO, stanem wydzielniczym osób uzależnionych od żucia tytoniu i potencjalnie złośliwymi zaburzeniami funkcjonowania jamy ustnej (OPMD). Stan wydzielania śliny zbadano u 100 osób. Odnotowano historię kliniczną i nawykową. Grupa I – 50 osób zdrowych z grupy kontrolnej; grupa II – 50 osób z nawykiem żucia tytoniu, z PMD lub bez. Z 1 ml śliny pobranego od każdego uczestnika badań wykonano test aglutynacji Wienera, aby poznać grupę krwi i status wydzielniczy. Związek między grupami krwi ABO, stanem wydzielniczym i OPMD oceniano za pomocą testu chi-kwadrat i ilorazu szans. Grupa kontrolna (I) miała grupy krwi A (44%), B (32%), O (14%), AB (10%), a grupa z nawykiem żucia tytoniu (II) miała A (32%), B (26%), O (34%), AB (8%). Stan niewydzielniczy w grupie I wynosił 18%, a w grupie II 82%. W grupie II 20% osób niewydzielających wykazywało PMD, głównie zwłóknienie podśluzówkowe jamy ustnej, a następnie leukoplakię i liszaj płaski. Wyraźna korelacja między stanem wydzielania śliny a rozwojem PMD wskazuje, że osoby niewydzielające mają większe ryzyko rozwoju zmian w jamie ustnej. Wśród osób z nawykiem żucia tytoniu grupy krwi B i O były bardziej zagrożone niż A i AB.

## Introduction

Habituals are individuals who indulge in habits such as chewing tobacco, areca nut with or without betel quid, gutkha, or tobacco smoking. In India, ease of access, unawareness among the individuals on the effects of tobacco chewing and the cost-effective marketing of tobacco-related products have led to an increase in the rate of oral potentially malignant diseases and cancer**.** The oral potentially malignant disorders (OPMDs) include both potentially malignant lesions and potentially malignant conditions. OPMDs are leukoplakia, oral submucous fibrosis (OSF), oral lichen planus (OLP), oral lichenoid lesions (OLL), actinic cheilitis, some inherited cancer syndromes and immunodeficient conditions (Rai, Acharya, Hallikeri, 2015). Among these, OSF has the highest rate of malignant transformation, being 7–13% followed by leukoplakia: 0.13 to 17.5%, OLP: 1.37%, and OLL: 2.34% (Amagasa, Yamashiro, Uzawa, 2011; Giuliani et al., 2019).

ABO blood grouping was given by Landsteiner and later in 1902 his pupils Von Decastallo and Sturli described the fourth blood group, AB (Metgud, Khajuria, Mamta, Ramesh, 2016). The literature review shows the correlation between ABO blood grouping and PMDs. Kumar, Puri, Laller, Bansal and Malik (2014) reported that blood group A patients had 1.28 times increased chances of developing OLP, followed by AB, B, O. In a study by Hallikeri, Udupa, Guttal and Naikmasur (2014) it was noted that individuals with blood group A had greater prevalence of OSF when compared to those with blood group O, B, AB. Studies in the Indian population have revealed patients with blood group A have a predisposition for oral cancer compared to other blood groups (Tyagi, Pradhan, Agarwal, 1965; Raaghavan, Bailoor, Jhansirani, 1986; Jaleel, Nagarajappa, 2012).

Besides blood, antigens are also secreted in various body secretions such as saliva, semen, gastric juice, nasal secretions, sweat, tears, urine, etc (Kumar et al., 2014). These individuals who secrete their blood type antigens in their body fluids are known as “secretors” and those who do not secrete their blood type antigens in their body fluids are known as “non-secretors”, accounting for 20% of population. The secretor gene, fucosyltransferase 2, is inherited in an autosomal

dominant pattern (Rai, Acharya, Hallikeri, 2015; Hallikeri et al., 2014). According to literature reviews, non-secretors are more prone to develop PMDs and cancer. Studies on the relationship between secretor status and premalignant and malignant oral lesions have shown a possible correlation amongst these factors. Rai et al. (2015) showed significantly more premalignant lesions in non-secretors. Study by Hallikeri et al. (2014) revealed an increased frequency of a premalignant condition, OSF in non-secretors. Vidas et al. (1999) studied the effect of the secretor status on oral precancerous lesions and noted that salivary non-secretors were at a higher risk of developing oral cancer compared to secretors.

Hence, with this background, we aimed to study the relationship between ABO blood group and secretor status among habituals. The aim was to analyse the ABO blood group and secretor status using saliva among habituals and healthy controls and to assess the relationship between ABO blood group, secretor status of habituals and OPMDs.

## Material and Methods

The source of data was obtained from patients reporting to the Department of Oral Medicine and Radiology and was conducted at a 2-month interval from November 2019 to December 2019. Ethical clearance was obtained from the Institutional Review Board (IRB. NO: 2019/HS/OP/69). The procedure was explained and informed consent was obtained from the participants of the study.

Salivary secretor status in 100 individuals was studied. After noting a detailed clinical and habit history the subjects were equally grouped as: a. Individuals having habit with or without potential malignant disorders (Group 1); b. Healthy controls (Group 2).

### Establishing the secretor status in the saliva

A sterile container was used to collect 1 ml of unstimulated saliva from each patient. This was transferred to a test tube, closed with cotton plug and kept in boiling water bath for 10 minutes to kill enzymes. The supernatant was then extracted by centrifugation with a force of 1,700 rpm for 10 minutes. Wiener agglutination test was used to analyse the secretor status. Test serum utilized in this experiment was diluted in a salted physiological solution in a proportion of 1 : 10, the same proportion that the saliva was diluted.

The test tubes were marked I to IV and the following antiserum was added into the test- tubes:

In both test tube I and II, 1 drop of saliva was taken followed by addition of 1 drop of anti-B serum and anti-A serum respectively. Whereas in test tubes III & IV, 1 drop of physiological solution was taken and 1 drop of anti-B serum and 1 drop of anti-A serum was added, respectively.

A drop of 2–3% suspension 'A' erythrocyte into sterile tube II and IV and a drop of suspension 'B' erythrocyte into tube I and III was added after 10 minutes. Following this the test tubes were agitated and kept at room temperature for one hour. The results were then noted.

Agglutination in tube I was due to substance A2 in saliva, i.e. of secretor A, and the agglutination in tube II was an evidence of secretor B. The lack of agglutination in tubes I and II designated AB secretor, and agglutination in tubes, I and II indicated the individual is a non-secretor.

### Statistical analysis

Chi-square test and odds ratios (OR) were applied to assess the relationship between ABO blood groups, secretor status, and OPMDs.

## Results

In this case-control study, a total of 100 participants, consisting of 50 controls including 7 females and 43 males; and 50 patients with habits including various forms of tobacco and areca nut habits were studied.

The frequencies of A, B, AB and O in the control group were 44%, 32%, 10%, and 14% respectively. In the habituals group, out of 50, blood group O predominated being 34%, followed by blood group A – 32%, B – 26% and the least was AB – 8%. A statistically significant difference was noted with blood group O among controls and habituals (Table 1). The present study reveals blood group O to be at a higher risk with an odds ratio of 2.43, followed by B, AB and least was A with OR of 0.81, 0.8 and 0.73 respectively at the CI of 95% (Table 2). The habits noted were mixed habit (48%), which predominantly included a combination of betel quid chewing with smoking or alcohol. Other habits were betel quid chewing (30%), tobacco chewing (16%) and smoking (6%) (Table 3).

Table 1. Distribution of ABO blood groups among controls and habituals

| Group | Blood gp A % (N) | Blood gp B % (N) | Blood gp AB % (N) | Blood gp O % (N) | Total percent % (N) |
|---|---|---|---|---|---|
| Controls | 44 (22) | 32 (16) | 10 (5) | 14 (7) | 100 (50) |
| Habituals | 32 (16) | 26 (12) | 8 (4) | 34 (17) | 100 (50) |
| p value | 0.33 | 0.57 | >0.99 | 0.04* | |

* Statistically significant ($p < 0.05$).

Table 2. Odds ratio blood group among individuals with habits

| Blood groups | OR (CI) |
|---|---|
| A | 0.73 (0.34–1.55) |
| B | 0.81 (0.35–1.86) |
| AB | 0.8 (0.2–3.15) |
| O | 2.43 (0.93–6.37) |

OR = Odds Ratio.
CI = Confidence Interval.

Table 3. Different habits among the habituals and their frequency

| Type of habit | Frequency | Percent |
|---|---|---|
| Betel quid chewing | 15 | 30.0 |
| Tobacco chewing | 8 | 16.0 |
| Smoking | 3 | 6.0 |
| Mixed | 24 | 48.0 |
| Total | 50 | 100.0 |

The secretor status was as follows: in the control group 82% were secretors and 18% were non-secretors, in contrast among the habituals 82% were non-secretors and 18% were secretors with a highly significant statistical difference ($P < 0.001$) (Table 4). Among the habituals, PMDs were seen in 10 out of 50 individuals, accounting to 20% and most frequent being OSF (7 (70%)), followed by one (10%) case each of OSF+ leukoplakia, lichen planus and leukoplakia (Table 5).

Table 4. Secretor status among the control and habituals

| Groups | Secretor | | Non-secretor | |
|---|---|---|---|---|
| | **N** | **%** | **N** | **%** |
| Control | 41 | 82 | 9 | 18 |
| Habituals | 9 | 18 | 41 | 82 |
| p value | <0.001* | | <0.001* | |

* Statistically highly significant ($p < 0.05$).

Table 5. Potentially malignant disorder among habituals

| Percentage of potentially malignant disorder among habituals | |
|---|---|
| Oral potentially malignant disorder present N (%) | Oral potentially malignant disorder absent N (%) |
| 10 (20) | 40 (80) |
| Distribution of potentially malignant disorder among habituals | |
| Oral potentially malignant disorders | Number of cases N (%) |
| Oral submucous fibrosis | 7 (70) |
| Lichen planus | 1 (10) |
| Leukoplakia | 1 (10) |
| Oral submucous fibrosis with leukoplakia | 1 (10) |

## Discussion

Blood groups are the identity of any individual and once determined will remain unchanged lifelong. Blood group can be determined with accuracy from saliva in individuals who secrete antigens. H antigen is the precursor of blood groups and present in all types of blood group. H antigen gets converted into blood groups A & B with the help of glucosyltransferase enzyme, whereas in individuals with O blood group, blood antigen remains the same in its original form. Aberration in the synthesis of glycolipids brings about down-regulation or partial or complete deletion of the blood group antigen on the cell surface, making it prone to disease development (Dabelsteen, Pindborg, 1973; Auclair, 1984).

In the present study, among the controls, predominant blood group noted was A (44%) and least was AB (10%), whereas among habituals blood group A (32%) & O (34%) were the major blood group noted. A statistically significant difference was noted with blood group O among both the groups. Further blood group O was at higher risk with odds ratio of 2.43, followed by B, AB and least was A with OR of 0.81, 0.8 and 0.73 respectively at the CI of 95%. Among the PMDs, predominantly seen were OSF (7 cases i.e, 70%) and one case (10%) each of OSF with leukoplakia, lichen planus and leukoplakia (total 3 cases). These PMDs were noted in blood group O individuals followed by blood group A, B & AB.

Similarly, Rai et al. (2015) noted PMDs in blood groups as follows: A – 42%, O – 39%, B – 9% and AB – 4%; with OR of 1.118 (0.67–1.86) and 1 (0.52–1.91) for blood group A and O respectively. Among the PMDs, predominantly noted was OSF and was seen among the blood group A individuals. A study by Hallikeri et al. (2014) showed that when compared to O, B, AB blood groups, the prevalence of OSF was more in those belonging to blood group A. However, a statistically significant relation could not be established between the blood groups and OSF. We noted only one case of OLP in those with blood group B. Moshaverinia, Rezazadeh, Dalvand, Moshaverinia and Samani (2014) found no statistically significant correlation between ABO blood groups and OLP. Whereas Kumar et al. (2014) found a significant association between blood group A and OLP on comparing cases and controls.

Habits are the possible factors for the occurrence of OPMD and oral cancer. Individuals who do not secrete antigens into body fluids are called non-secretors, and are more prone to disease development as reported by Rai et al. (2015) and Lamey et al. (1991). In addition to the habit, the non-secretor status decreases the susceptibility of mucosal resistance to infection and pathological changes (Lamey et al., 1991; Rai et al., 2015).

In the present study, the control group had 82% secretors and 18% non-secretors and among the habituals, 18% were secretors and 82% non-secretors with a statistically significant difference. Vidas et al. (1999) studied secretor status of saliva in oral precancerous lesions and observed maximum number of oral disease and also epithelial dysplasia in the non-secretor group. Hallikeri et al. (2014) in a study on salivary secretor status in OSF patients found all OSF patients were non-secretors and concluded that non-secretors are at a greater risk of developing of oral lesions than the secretors. Rai et al. (2015) noted a significant statistical association between the control and study groups with salivary secretor status. The 87% of the OPMDs were non-secretors, whereas in the control group, the non-secretor status percentage was 16%. And similar to the present study, OSF and leukoplakia patients examined were non-secretors. Pourazar, Joshi, Sathe, Advani and Bhatia (1986) studied group of 100 leukoplakia patients and reported that majority were non-secretors when compared to the healthy group. Campi et al. (2007) found no significant relationship between oral lesions (malignant and premalignant) and secretor status ($P = 0.119$).

In their study, Bakhtiari, Far, Alibakhshi, Shirkhoda and Anbari (2019) reported that of the oral carcinoma patients, 66.7% were non-secretors, whereas among the controls, 73.6% were secretors. A statistically significant difference was noted between the groups.

The salivary secretor status in chronic hyperplastic candidosis patients was investigated by Lamey et al. (1991). It was suggested that secretor status is a genetically determined risk marker in the potentially malignant oral lesion of candidal leukoplakia. The protective mechanism of the blood group antigens against the infections is by interfering with the bond between the epithelial cells and microorganisms. Also, lower levels of IgA have been noted in the serum and saliva of non-secretors. Hence, the specific immune responses of the mucosa among the non-secretors are weaker than secretors (D'Adamo, Kelly, 2001).

## Conclusion

To conclude our study revealed blood groups O and B were more susceptible to OPMD development. Among OPMDs, most commonly noted was OSF, which has a high rate of malignant transformation. Salivary non-secretor status among habituals makes individuals, more prone to OPMDs. Counselling the habituals to quit the habit and close follow-up of non-secretor individuals can cause early detection and prompt treatment of oral potentially malignant disorders.

## Acknowledgements

The authors would like to thank Mrs. Ambika Patil, SDM College of Dental Sciences & Hospital, Dharwad, for the laboratory assistance in examining the salivary secretary status.

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**Cite as:** Iyer, S., Hallikeri, K., Monteiro, R., Burde, K. (2021). Study of ABO blood grouping and secretor status among habituals – A case control study. *Acta Biologica*, *28*, 33–40. DOI: 10.18276/ab.2021.28-04.