

## ORIGINAL ARTICLE

# Assessment of ABO blood grouping and secretor status in the saliva of the patients with oral potentially malignant disorders

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**ABSTRACT**

**Background:** Secretor status may possibly be one of the factors in the etiopathogenesis of oral precancerous lesions and subsequently cancer. Studies have shown the relationship between the pathogenesis of disease and secretor status. They have made known that secretor status is a possible factor influencing disease status. Studies have revealed the association between blood groups and specific diseases. **Aims:** To assess any association of ABO blood grouping with oral potentially malignant disorders (OPMDs) and to examine whether there is any difference in the saliva secretor status in the patients with OPMDs and healthy controls. **Materials and Methods:** The study consisted of 90 subjects, with 45 patients assigned to two groups (a) Patients with potentially malignant disorders and (b) healthy controls. ABO blood grouping was done and 1 ml of unstimulated saliva was collected in a sterile test tube. The Wiener agglutination test was performed to analyze the secretor status in both the groups. Chi-square test and odd ratio were used to assess the relationship between ABO blood group and OPMDs. Chi-square test was performed to assess the relationship between secretor status and OPMDs. Probability level was fixed at <math><0.05</math>. **Results:** The results demonstrated a statistically significant relation between OPMDs and secretor status ( $P = 0.00$ ). Eighty-seven percent of patients with OPMDs were nonsecretors, while in the control group sixteen percent of them were nonsecretors. There was no statistically significant relationship between ABO blood groups and OPMDs ( $P > 0.05$ ). **Conclusions:** The study confirms the inability to secrete blood group antigens in the saliva of patients with OPMDs which could be regarded as a host risk factor. Results could not propose a relationship between ABO blood group and OPMDs.

**Key words:** ABO blood grouping, oral potentially malignant disorders, secretor status

## INTRODUCTION

In a recently held World Health Organization workshop, it has been recommended to abandon the distinction between potentially malignant lesions and potentially malignant conditions and to use the term oral potentially malignant disorders (OPMD) instead. OPMD includes leukoplakia, erythroplakia, oral lichen planus (OLP), oral submucous

fibrosis (OSF), actinic cheilitis, some inherited cancer syndromes and immunodeficient conditions.<sup>[1]</sup>

Alexzender in 1921 was first to describe the possibility of an association between ABO blood groups and malignancy. Since then, a number of studies were carried out to establish this

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correlation.<sup>[2]</sup> Tyagi *et al.*, revealed, in the Indian population, that patients with blood group A have a predisposition for oral cancer.<sup>[3]</sup> Jaleel and Nagarajappa demonstrated similar results in their study with increased risk of oral cancer associated with blood group A.<sup>[4]</sup> Kumar *et al.*, found that patients with blood group A had 1.28 times higher risk of developing OLP, followed by AB, B, O.<sup>[2]</sup> Hallikeri *et al.*, observed that OSF was more prevalent among those with blood group A compared to O, B, AB.<sup>[5]</sup> Thus, we plan to assess the association of ABO blood grouping with OPMD as this emerged as less investigated area based on the literature search.

At present, the most common method for ABO blood typing is from a drop of blood obtained by pricking the finger. Besides blood, the antigens are also secreted in various other body secretions, such as saliva, semen, gastric juice, nasal secretions, sweat, tear, urine etc, from which blood groups can be determined.<sup>[6]</sup> The saliva, as well as other body liquids of about 70–80% of the population contains ABO (H) blood group antigens. Their secretion is under the control of the secretory gene.<sup>[7]</sup> Some people possess the ability to secrete blood group substances in the saliva and they are referred to as “secretors”, whereas others lack such an ability and are referred to as “non-secretors”.<sup>[8]</sup> It has been reported that nonsecretor individuals are more prone to develop lesions and certain types of cancers are more prevalent in particular blood group types.<sup>[5]</sup> It has been confirmed in a number of earlier studies on the etiology and pathogenesis of certain diseases that the patients’ secretor status [ABO (H) blood group antigens] may possibly be a factor influencing the development of systemic oral diseases.<sup>[7]</sup> This possibility has instigated the present study.

### Objectives

- To assess the relationship between ABO blood grouping and OPMDs
- To evaluate if any of the ABO blood groups is associated with an increased risk for OPMDs
- To study the difference in the saliva secretor status in the patients with OPMDs and healthy controls.

### MATERIALS AND METHODS

The source of data for this cross-sectional study was patients reporting to the Department of Oral Medicine and Radiology and was conducted at a 3-month interval between January 2014 and April 2014. An ethical clearance was obtained from the Institutional Review Board (IRB.NO: 2014/P/OP/20). Informed consent was obtained from the participants of the study. The study consisted of 90 subjects, with 45 participants assigned to two groups; (a) Patients with potentially malignant disorders and (b) Healthy controls.

Patients were clinically examined for the OPMDs and clinically confirmed cases of OSF, OLP and leukoplakia

were included. Suspicious cases were subjected to biopsy and histopathological examination to confirm the clinical diagnosis. Sample collection included blood for ABO grouping and 1 ml of unstimulated saliva in a sterile test tube to assess the secretor status by Wiener agglutination test. Systemically healthy participants were included in the study. Those patients diagnosed with any premalignancy or malignancy and undergoing treatment for the same were excluded. The control group consisted of age- and sex-matched healthy volunteers.

### Methodology

ABO blood grouping was done in the Department of Clinical Hematology for all the participants of the study. The procedure for assessment of the secretor status in saliva was followed as per Vidas *et al.*,<sup>[7]</sup> a total of 1 ml unstimulated saliva was collected from each participant and stored in a sterile glass vial. The saliva was then poured into a sterile test tube and closed with a cotton plug. The test tube was then placed in a boiling water bath for approximately 10 min to destroy the enzymes [Figure 1]. The supernatant fluid was then extracted in a centrifuge at 1700 rpm for 10 min [Figure 2]. The Wiener agglutination test was used to establish the secretor status [Figure 3]. The test serum was diluted in a salted physiological solution of 1:10 dilution. The following antisera were then placed in test tubes marked I–IV: (a) I, one drop of saliva + one drop of anti B serum; (b) II, one drop of saliva + one drop of anti A serum; (c) III, one drop of physiological solution + one drop of anti B serum; and (d) IV, one drop of physiological solution + one drop of anti A serum. After 10 min at room temperature, one drop of 2–3% of suspension “A” erythrocytes was added into sterile tubes II and IV and one drop of suspension “B” erythrocytes into tubes I and III. All the test tubes were agitated and left at room temperature. Readings were available after 1-hour. Test tubes III and IV (controls) showed agglutination. Agglutination in tube I was as a result of the presence of substance A2 in saliva, that is, of secretor A, while the agglutination in tube



**Figure 1:** Test tube containing unstimulated saliva sample placed in boiling water bath for approximately 10 min to destroy enzymes

II was proof of secretor B. The absence of agglutination in tubes I and II designated a AB secretor status and at the same time agglutination in tubes I and II [Figure 4] has proven that the person is non-secretor. This is because AB secretor will have both the antigens in the saliva which will interfere with

agglutination in tubes I and II due to utilization of the antiserum prior to the addition of erythrocytes. Non-secretors have no antigen in the saliva, so tube I and II shows agglutination simultaneously, similar to tube III and IV.



Figure 2: The sample centrifuged for 10 min at 1700 rpm to obtain the supernatant fluid

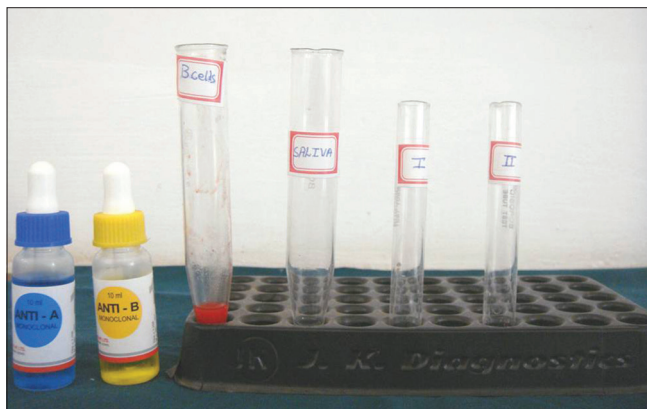


Figure 3: Wiener agglutination test



Figure 4: Agglutination in both tubes I and II indicating nonsecretor status

Statistical analysis

Chi-square test and odds ratio were applied to assess the relationship between ABO blood groups and OPMDs. Chi-square test was performed to assess the relationship between secretor status and OPMDs.

RESULTS

Table 1 depicts the frequency of OPMDs in different age groups. Table 2 shows the distribution of ABO blood groups among the cases and controls. Out of 45 OPMDs, 19 (42%) had blood group A, 9 (20%) had B, 4 (9%) had AB and 13 (29%) had O. The frequencies of blood types A, B, AB and O were 38, 29, 4 and 29%, respectively, among control participants. When cases and controls were compared, no significant difference was found between blood group and OPMDs ( $P > 0.05$ ).

Table 3 shows that when the strength of association between ABO blood groups and OPMDs was assessed by

Table 1: Frequency of OPMDs in different age groups

Age	OPMDs
16-25	11
26-35	10
36-45	8
46-56	7
56-65	5
66-75	4
Total	45

OPMDs: Oral potentially malignant disorders

Table 2: Blood group characteristics among OPMD patients and controls

Subjects	Blood group (%)				Total (%)
	A	B	AB	O	
With OPMDs	19 (42)	9 (20)	4 (9)	13 (29)	45 (100)
Controls	17 (38)	13 (29)	2 (4)	13 (29)	45 (100)
<i>P</i>	0.667	0.327	0.398	1	

Chi-square test; OPMDs: Oral potentially malignant disorders

Table 3: OR showing strength of association between ABO blood groups and OPMDs

Blood groups	OR (CI)
A	1.118 (0.67-1.86)
B	0.692 (0.33-1.405)
AB	2 (0.39-10.4)
O	1 (0.52-1.91)

OPMD: Oral potentially malignant disorders, OR: Odds ratio, CI: Confidence interval

odds ratio, results were inapt to comment, as the sample size was small.

Figure 5 shows the frequency distribution of experimental group (OPMDs). Out of 45 patients with OPMDs: 24 were with OSF; 13 with OLP and 8 with leukoplakia. Twenty-nine (64%) are below 30 years and 16 (36%) were above 30 years. Forty (89%) were males and 5 (11%) females. Table 4 shows the type and distribution of tobacco – related habits in patients with OPMDs. Thirty-seven (82%) patients with OPMDs were tobacco chewers using gutka or pan masala.

In the present study, 32 cases and 32 controls of A, B and AB were analyzed for the saliva secretor status. The procedure followed checks the presence or absence of A or B substance in the saliva. Group O individuals with no secretor A or B substance secrete more H than do persons of group A or B, hence were not included. The secretor status of patients with OPMDs is shown in Figure 6 and the secretor status of the healthy control group is shown in Figure 7. Eighty-seven percent (28) were nonsecretors and 13% (4) were secretor of the 32 subjects examined in the experimental group (OPMDs) whereas in the control group 16% (5) were nonsecretors and 84% (27) were secretors. The difference between the experimental and control groups with regard to saliva secretor status were statistically significant ( $P = 0.00$ ) [Table 5].

Table 6 shows the relation of secretor status regarding gender. Twenty-four (75%) of males in OPMDs were nonsecretors whereas 4 (12.5%) males in the control group were nonsecretors. A statistically significant difference between secretors and nonsecretors having OPMDs was found with regard to the gender ( $P = 0.00$ ). Table 7 shows experimental group patients’ with OPMDs involving multiple sites; 0 (0%) were secretors and 12 (37.5%) nonsecretors, in cases of single site involvement, 4 (12.5%) were secretors and 16 (50%) were nonsecretors. In the experimental group, all patients with OSF 18 (56%) and leukoplakia 5 (16%) were nonsecretors. In case of OLP, 5 (16%) were nonsecretors and 4 (12.5%) were secretors.

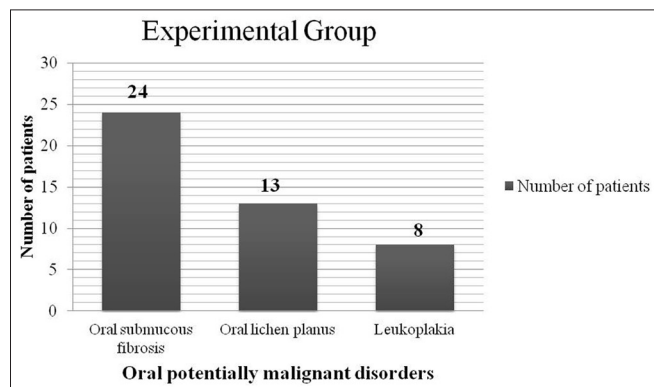


Figure 5: Distribution of patients with oral potentially malignant disorders

## DISCUSSION

The ABO system is the most investigated erythrocyte antigen system.<sup>[8]</sup> The association between blood groups and different diseases such as various cancers, diabetes mellitus, skin disease, heart disease, genetic disease and dental caries, had been studied. Some of this research had shown elevated relative risks for some diseases. However, there are no uniform results.<sup>[9]</sup>

Studies done by Tyagi *et al.*,<sup>[3]</sup> Jaleel and Nagarajappa<sup>[4]</sup> have shown that individual with blood group A have a predisposition for oral cancer. Kumar *et al.*, found a significant relationship between blood group A and OLP when cases and controls were compared.<sup>[2]</sup> Hence, this study was conducted to evaluate blood groups in patients with OPMDs that could provide useful information about the risk factors of this condition among Indians residing in this region. Out of 45 OPMDs, 19 (42%) had blood group A, 9 (20%) had B, 4 (9%) had AB and 13 (29%) had O. The frequencies of blood types A, B, AB and O were 38, 29, 4 and 29%, respectively, among control participants. When cases and controls were compared, no significant difference was found between blood group and OPMDs ( $P > 0.05$ ). Similar to the present study, Moshaverinia *et al.*, found no statistically significant relationship between ABO blood groups and OLP.<sup>[9]</sup> Hallikeri *et al.*, could not establish a statistically significant relationship between ABO blood groups and OSF.<sup>[5]</sup>

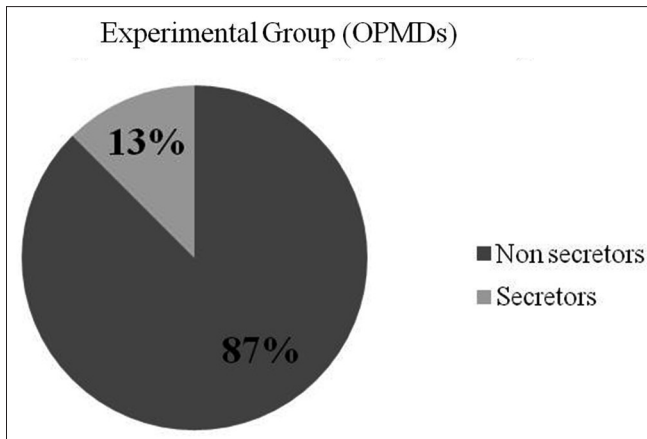
Betel quid chewing, tobacco smoking, alcohol, nutritional status, gender and age are the important factors associated with increased risk of oral cancer. A considerable number of oral cancers develop from preexisting lesions such as leukoplakia, erythroplakia and OSF. Accordingly attempts have been made to investigate host risk factors involved in the development of oral potentially malignant lesions. One such candidate to mark the risk of susceptibility to disease is the patient’s secretor status.<sup>[10]</sup>

Secretor status of an individual is genetically determined by a pair of allomorphic genes: Se and se with Se dominant over se. As a consequence, individuals who are either homozygous Se-Se or heterozygous Se-se are secretors while homozygous se-se are nonsecretors. The dominant Se allele regulates the expression of H transferase in certain glands and secretions. The red cells and plasma of nonsecretors fully express the

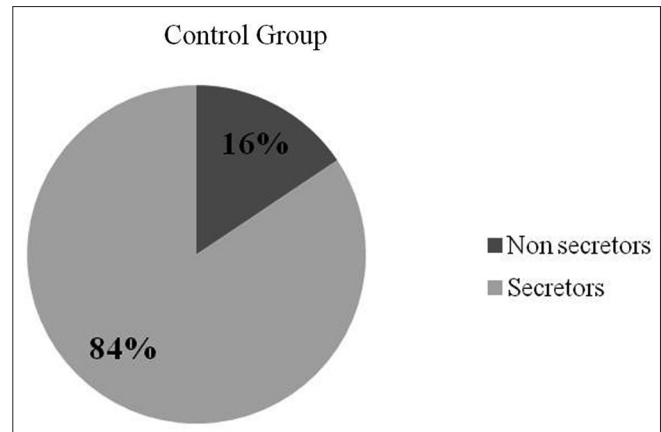
Table 4: Type of tobacco related habit and distribution in patients with OPMD

Habits	Frequency (%)
Tobacco chewing	37 (82)
Tobacco smoking	2 (4)
Combination of habits	6 (13)
Total	45 (100)

OPMD: Oral potentially malignant disorder



**Figure 6:** The secretor status of the 32 subjects examined within in the experimental group (oral potentially malignant disorders) which consisted 87% (28) nonsecretors and 13% (4) secretors



**Figure 7:** The secretor status of the 32 subjects examined within in the control group which consisted of 16% (5) nonsecretors and 84% (27) secretors

**Table 5: Saliva secretor status among OPMDs patients and controls**

Groups	Nonsecretor (%)	Secretor (%)	Total (%)
OPMDs	28 (87)	4 (13)	32 (100)
Controls	5 (16)	27 (84)	32 (100)
<i>P</i>	0.00	0.00	

Chi-square test; OPMDs: Oral potentially malignant disorders

**Table 6: Relation of secretor status regarding gender**

Gender	Experimental group (OPMDs) (%)		Control group (%)	
	Nonsecretors	Secretors	Nonsecretors	Secretors
Men	24 (75)	4 (12.5)	4 (12.5)	11 (34)
Women	4 (12.5)	0 (0)	1 (3)	16 (50)
Total	28 (87.5)	4 (12.5)	5 (16)	27 (84)
<i>P</i>	0.00		0.00	

Chi-square test; OPMD: Oral potentially malignant disorder

**Table 7: Relation of secretor status and site in patients with OPMD**

Number of sites	Secretors (%)	Nonsecretors (%)
Single	4 (12.5)	16 (50)
Multiple	0 (0)	12 (37.5)
Total	4 (12.5)	28 (87.5)
<i>P</i>	0.098	

Chi-square test; OPMD: Oral potentially malignant disorder

specifications determined by their H and ABO genes, but the saliva and other tissue fluids contain no H transferase and, therefore, no A, B and H substance. A, B, H substances in the secretions are influenced by ABO type. All secretors have H antigen in their secretions. O Group individuals with no secretor A or B substances secrete more H than do persons of group A or B. Around 80% of the normal population are secretors and nonsecretors are susceptible to a variety of infective conditions.<sup>[10]</sup> The ability of secreting the blood

group antigens plays a significant role in the natural resistance of the organism to infections.<sup>[7]</sup>

Lamey *et al.*, investigated the secretor status in the saliva of chronic hyperplastic candidosis patients and suggested that secretor status is a genetically determined risk marker in the potentially malignant oral lesion of candidial leukoplakia.<sup>[11]</sup> Similar to above-mentioned study, in the present study, the difference between the experimental and control groups with regard to the secretor status was statistically significant (*P* = 0.00). Eighty-seven percent of those having OPMDs were nonsecretors, whereas the percentage of nonsecretors in the control group was statistically significantly lower (16%). A study on the secretor status in the saliva of patients with oral precancerous lesions by Vidas *et al.*, recorded a higher intensity of oral disease in the nonsecretor group and the occurrence of epithelial dysplasia was found exclusively in the nonsecretor group.<sup>[7]</sup> In an analysis of salivary secretor status in patients with OSF by Hallikeri *et al.*, they found that all of the OSF patients were nonsecretors. They concluded that nonsecretors are at a greater risk and are more prone to the development of oral lesions. There is a correlation between salivary secretor status and the development of OSF.<sup>[5]</sup> All OSF and leukoplakia patients examined in the present study were nonsecretors. The results of present study are similar to results of Pourazar *et al.*, who examined a group of 100 patients with leukoplakia, established an increased number of nonsecretors in the diseased group.<sup>[12]</sup>

In a study on examining the presence of ABO (H) antigens of the blood types in the saliva of patients with oral cancer by Cerovic *et al.*, could not confirm the hypothesis that nonsecretors are more prone to the development of oral cancer.<sup>[8]</sup> Lamey *et al.*, investigated the secretor status in the saliva of a group of Sri Lankan patients and found that secretor status does not appear to be an associated risk marker for the development of oral cancer.<sup>[10]</sup> Vidas *et al.*, examined the influence of secretor status on certain oral precancerous lesions and stated that an

inability to secrete blood group antigens in saliva could be regarded as a risk factor in the development of cancer.<sup>[7]</sup>

The present study was a pilot study and the first of its kind to assess the saliva secretor status and ABO blood grouping in individuals with OPMDs. However, future studies with larger sample sizes are required to validate the study results. Secretor status can be part of routine investigations to assess the disease status and to check an individual's susceptibility to manifest the disease.

## CONCLUSIONS

OSF was the most commonly reported OPMDs in this region. This can be attributed to the chronic gutka and pan chewing habit practiced in this area. Observations of frequency distribution suggested that OPMD was more prevalent among those with blood group A compared to O, B, AB. However, the result could not suggest a relationship between ABO blood group and OPMDs. This can be attributed to the small sample size examined. According to the results, ABO blood groups are not a risk factor for OPMDs. The results demonstrated a statistically significant relation between OPMDs and secretor status. The study confirms that the inability to secrete blood group antigens in the saliva of patients with OPMDs, could be regarded as a host risk factor.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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