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# Salivary Secretor Status of Blood Group Antigens in Patients with Head and Neck Cancer

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## Abstract

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**Keywords:** Head and neck cancer; Secretor status; Blood group antigens

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**BACKGROUND:** Head and neck cancers include malignancies of the scalp and neck skin, nasal cavity, paranasal sinuses, oral cavity, salivary glands, pharynx and larynx. The term ABO secretor refers to people who secrete blood group antigens in their body fluids such as saliva, sweat, tears, semen, and serum. Non-secretors refer to those who do not secrete their blood group antigens in their body fluids. The lack of blood type antigens in body discharge increases the susceptibility to certain types of diseases and infection.

**AIM:** Our study aimed to investigate the relationship between the secretion of blood groups in the saliva of patients with head and neck cancers.

**MATERIAL AND METHODS:** This case-control study was performed on 110 people (57 patients with head and neck cancer who were referred to Imam Khomeini Hospital, Tehran and 53 cancer-free controls). Five ml of non-stimulated saliva were collected by the spitting method. By agglutination or lack of agglutination in the test tubes, we determined the patient's secretor or non-secretor condition.

**RESULTS:** In terms of secretor status, 52.7% of all samples were secretors. In the case group, 19 out of 57 cases (33.3%) were secretors, and 38 were non-secretors (66.7%). In the control group, 39 out of 53 cases (73.6%) were secretors, and 14 cases were non-secretors (26.4%). There was a significant difference in the percentage of non-secretors between the two groups ( $p = 0.00$ ).

**CONCLUSION:** People with non-secretor status may be more prone to develop head and neck cancer. The presence of these antigens in saliva may have a protective effect.

## Introduction

Head and neck cancers include malignancies of the scalp and neck skin, nasal cavity, paranasal sinuses, oral cavity, salivary glands, pharynx and larynx [1], considered the sixth most common cancer in the world, accounting for 10% of malignancies worldwide and 3% of malignancies in the United States [2], [3], [4], [5], [6].

Tobacco and alcohol are two major risk factors for head and neck cancers, especially squamous cell carcinoma and the probability increases with higher intake [7], [8]. Also, researches have shown the association of other agents, such as

human papilloma virus and blood group antigens with cancer development [9], [10], [11], [12].

The term ABO secretor refers to people who secrete blood group antigens in their body fluids such as saliva, sweat, tears, semen, and serum, and non-secretors refer to those who do not secrete their blood group antigens in their body fluids [13], [14]. The secretor gene (fucosyltransferase 2) is inherited in the autosomal dominant pattern: Se is the dominant form, and se is the recessive form. Therefore, Se Se or Se se we are secretors, and se se we are non-secretors [15]. According to studies, the lack of blood type antigens in body discharge is considered a limitation, as it increases the susceptibility to certain types of diseases, including peptic and duodenal ulcers,

periodontal disease and candidiasis [16], [17], [18], [19], [20], [21], [22].

The protective mechanism of the blood group antigens against a variety of infections is unclear, but it seems that it interferes on the bond between the microorganism and epithelial cells, because many bacteria show a reciprocal response to blood group antigens. Also, in non-secretors, lower IgA levels were reported in both serum and saliva. Hence, it is likely that specific immune responses at mucosal surfaces of non-secretors are weaker than secretors. Lower levels of IgG have also been observed in these individuals [13].

Studies on the relationship between secretor status and diseases such as pre-malignant and malignant oral lesions have shown different results. Cerovic et al., (2008) and Lamey et al., (1994) found no significant relationship between secretor status and oral cancer [23], [24]. While Rai et al., (2015) showed significantly more pre-malignant lesions in non-secretors [25]. In Hallikeri et al. 's study (2015), oral mucosal fibrosis, a pre-malignant lesion, was more common in non-secretors [26]. As Jamil et al., reported (2005) it was shown that lack of secretion of blood group antigens in saliva could be considered as a risk factor for oral cancer [27].

Our study aimed to investigate the relationship between the secretion of blood groups in the saliva of patients with head and neck cancers.

## Material and Methods

This case-control study was performed on 57 patients with head and neck cancer who were referred to Imam Khomeini Hospital in Tehran (Cancer Institute), and their diagnosis was determined by histopathologic examination. Also, 53 patients' companions and hospital staffs were considered as the control group. Patients who were suffered from severe dry mouth for any reason, such as radiotherapy and those who were unable to give their saliva sample due to extensive oral surgery were excluded from the study.

First, the information checklist was completed based on patients' medical records, and then 5 ml of non-stimulated saliva were collected in a 15 ml falcon tube with a plastic cap between 9 am, and 12 am, while the patient was in sitting position, and then the tubes were placed in a boiling water bath for 10 minutes to denature the salivary enzymes. After that, the tubes were centrifuged for 10 minutes at 1700 rpm and the supernatant layer was removed and transferred to 2 ml micro tubes, kept in a freezer at -70°C until complete collection of the specimens.

According to Cerovic et al. 's study (2008),

secretor status was detected using the Inhibition Agglutination Test (Wiener Test) [23].

For each case, four test tubes were prepared, and "A" or "B" anti-sera were added as presented in table 1. Test tubes 3 and 4 were considered as control groups. We shook all the tubes at room temperature. After one hour, the readings were ready. By agglutination or lack of agglutination in the tubes, we determined their secretor or non-secretor state.

**Table 1: Content of test tubes**

	Content
Test tube 1	One drop of saliva + one drop of an anti-B+ suspension of RBC B type
Test tube 2	One drop of saliva + one drop of an anti-A+ suspension of RBC A type
Test tube 3	One drop of <i>physiological</i> saline +one drop of an anti-B+ suspension of RBC B type
Test tube 4	One drop of <i>physiological</i> saline + one drop of an anti-B+ suspension of RBC B type

In tubes 3 and 4, control tubes, agglutination occurred, and the results in tubes 1 and 2 were interpreted as follows:

- Lack of agglutination in tube 1 showed neutralising anti-B with salivary antigen, which meant that antigen B was present in saliva.
- Lack of agglutination in tube 2 showed neutralising anti-A with salivary antigen, which meant that antigen A was present in the saliva.
- Agglutination in tube 1 showed the reaction between Anti-B and B red blood cells. As a result, antigen B was not present in saliva.
- Agglutination in tube 2 showed the reaction between anti-A and "A" red blood cells. As a result, antigen A was not present in saliva.

So, no agglutination in tube 1 and agglutination in tube 2 meant: B secretor; agglutination in tube 1 and no agglutination in tube 2 meant: A secretor; lack of agglutination in both tubes 1 and 2 meant: AB secretor; agglutination in both tubes 1 and 2 meant: non-secretor. The secretor status was considered as a nominal variable, and the difference between the patients' group, compared to the healthy control group was assessed using the Chi-square test. The significance level was considered less than 0.05.

In this study, there was no interference in the patients' treatment process, and the condition was only described. However, to ensure patients' awareness and obtain their consent, the consent form was signed by all individuals and confirmed by the Ethics Committee with the code IR.SBMU.RIDS.REC.1396.479.

## Results

In this case-control study, a total of 110 people including 57 patients (25 female and 32 male) with a mean age of  $58.69 \pm 14.84$  years with head and

neck cancer and 53 controls (26 women and 27 men) with a mean age of  $49.13 \pm 11.13$  years participated. All patients in the case groupware admitted to the surgery department and Cancer Surgery Clinic of Imam Khomeini Cancer Institute.

In terms of smoking and alcohol in the case group, 17 out of 57 smoked (29.8%) and 2 consumed alcohol (3.5%); these two people were alcoholics and smokers at the same time.

There were 59 lesions in 57 patients, of which 39 cases were squamous cell carcinoma (SCC), 10 cases were basal cell carcinoma (BCC), 3 cases were sarcoïd cell carcinoma, 3 cases were mucoepidermoid carcinoma (MEC), 2 cases were osteosarcoma, 1 was pleomorphic adenoma, and 1 was Acinic-cell carcinoma (ACC). Meanwhile, one patient had two lesions in the tongue and buccal area, and another in mandible and tongue simultaneously. The total number of lesions was 59 in 57 patients.

The most frequent lesion site was tongue with 13 cases; other sites involvement were as follow: mandibular bone 9 cases, buccal mucosa 8 cases, facial skin 8 cases, parotid 5 cases, larynx 4 cases, lip 3 cases, maxillary bone 3 cases, nasal mucosa 3 cases, maxillary sinus 2 cases and one case had oral floor involvement.

The most common lesions were as amass (54.23%), and the rest (45.77%) were ulcerative. (Type of cancer, site, and shape are given in Table 2.)

**Table 2: Features of malignancies in head and neck cancers**

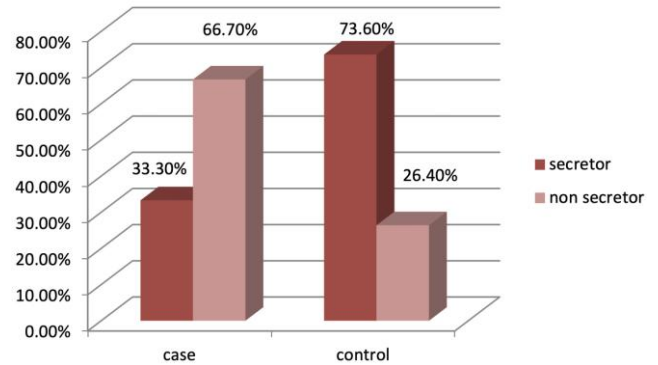
Variable	Description	Number	Percentage	
Cancer type	Squamous cell carcinoma	39	66.1%	
	Basal Cell Carcinoma	10	16.9%	
	Spindle cell sarcoma	3	5.1%	
	Mucoepidermoid carcinoma	3	5.1%	
	Osteosarcoma	2	3.4%	
	Pleomorphic adenoma	1	1.7%	
	Adenoid cystic carcinoma	1	1.7%	
	Total	59	100%	
	Anatomic location	Tongue	13	22.3%
Mandible		9	15.3%	
Buccal mucosa		8	13.5%	
Skin		8	13.5%	
Parotid		5	8.4%	
Larynx		4	6.7%	
Lip		3	5.1%	
Maxilla		3	5.1%	
Nose		3	5.1%	
Sinus		2	3.3%	
The floor of the mouth		1	1.7%	
Total		59	100%	
Clinical manifestation of the Lesions		Mass	32	54.23%
		Ulcer	27	45.77%
	total	59	100%	

In terms of lesions' size, among the total of 59 lesions, the smallest reported lesion was 5 mm, and the largest was 70 mm. The mean size was reported to be  $21.19 \pm 16.13$  mm.

Lymph node involvement was positive in 18 of 57 patients (31.6%). In the case group, 38 of 57 patients were new cases of cancer (66.7%), and 19 cases were diagnosed to have recurrent cancer (33.3%).

In terms of secretor status, 52.7% of all samples were secretors. In the case group, 19 out of

57 cases (33.3%) were secretors, and 38 were non-secretors (66.7%). In the control group, 39 out of 53 cases (73.6%) were secretors, and 14 cases were non-secretors (26.4%) (Figure 1).



**Figure 1: Secretory status in case and control groups**

There was a significant difference in the percentage of non-secretors between the two groups ( $p = 0.00$ ). In general, according to Wiener anti-agglutination test, 19 out of 57 patients were a secretor, among whom 12 were "A" secretor, 6 were "B" secretor, and one was "AB" secretor.

As mentioned above, the most common type of cancer among the case group was head and neck squamous cell carcinoma (HNSCC), 39 lesions (37 patients) among 59 lesions (66.1%). Therefore, the secretor status in patients with this type of cancer was analysed separately. In this group, 32.4% of subjects were secretors, and 67.6% were non-secretors. The difference between non-secretors in HNSCC and the control group was statistically significant (Table 2).

## Discussion

Blood group antigens can be present in physiological fluids such as saliva. The hypothesis that non-secretors are more likely to develop infections, malignant and pre-malignant oral diseases, chronic periodontitis and dental caries, led us to assess the secretor status of patients with head and neck malignancies [28], [29], [30].

Fucosyltransferase 2 is an enzyme synthesizes by the secretor gene. This enzyme adds fucose to the sugar moiety of glycolipids and glycoproteins. By Fucosyltransferase 2 on precursor oligosaccharides chain (type A) H is formed in secretions. By adding fucose to H, fucosyltransferase 3 can lead to the production of Le/b. Thus, all people whose phenotype consists of Le/b are secretors. Fucosylated oligosaccharides which exist at the cell surface participating in severe biological processes such as cell differentiation, cell movement and

adhesion. Saliva and other body secretions of ABH secretors contain considerable amounts of carbohydrates compared to non-secretors. This can lead to interference with previously mentioned functions [15].

In this study, 52.7% of the total population were secretors (33.3% of the case and 73.6% of the control group) with statistically significant difference ( $p < 0.001$ ). This means that people without blood group antigens in the saliva may be at increased risk for head and neck malignancies. Some previous studies have suggested a lack of secretion of blood group antigens in saliva as a risk factor for the development of oral epithelial dysplasia and malignant tumours [26].

It has been reported by Jamil et al., (2016) that 56% of the case group with oral cancer and 78% of the control group were secretors and there was a statistically significant relationship between secretor status and oral cancer ( $p < 0.01$ ) [20]. The result of this study was consistent with the present study. While in studies by Cerovic et al., (2008) and Lamey et al., (1998), assessment of blood group antigens in patients with oral cancer showed no significant difference between the two groups in terms of secretor status ( $P > 0.05$ ) [23], [24].

In Garrett's study (1971), there was no difference between the patients with benign and malignant salivary gland tumours and the control group in terms of secretor status [30].

Rai et al., (2015) evaluated the secretor status of the blood group antigens in oral premalignant lesions (leukoplakia, oral lichen planus, and subcutaneous fibrosis). In the case group, 87% of the subjects and in the control group only 16% were non-secretor, and there was a significant relationship between secretor status and pre-malignant oral lesions ( $p = 0.00$ ) [25]. These results are consistent with the present study. The results of Vidas and Temmer's study (1999) showed no significant difference in saliva secretor status of patients with premalignant oral lesions (lichen planus, leukoplakia, etc.) ( $p = 0.05$ ) meanwhile a higher intensity of these lesions was found in the non-secretor group ( $p = 0.01$ ) that indicates the progression of pre-malignant oral lesions in this group of patients [31].

In Campi et al. 's study (2007) there was no significant relationship between oral lesions (malignant and premalignant) and secretor status ( $p = 0.119$ ) [29].

Regarding oral submucosal fibrosis (OSMF) as a pre-malignant lesion, the study by Hallikeri et al., (2015) divided the samples into three groups. In the end, the results indicated that all members of the first group (tobacco users, with submucosal fibrosis) were non-secretors, in the second group (tobacco users, without oral lesions) 84% were non-secretors, and in the third group (healthy control subjects) 15% were

non-secretors. These results showed a statistically significant difference between the first and third groups, indicating the role of secretor status in the development of oral submucosal fibrosis [26].

In the study of Shahidi Dadars et al., (2016), most patients with lichen planus were non-secretors (74%), while in Bakhtiari et al. 's study (2016) on patients with lichen planus 84% of the cases and 80% of the control group were secretors, and there was no statistically significant difference between the patient and control groups in terms of secretor status (32) ( $P = 0.73$ ).

Another study by Lamey et al., (1991) showed that the percentage of non-secretors in the case group (68%) was significantly higher than the control group (38%) [21].

Lack of blood antigens in the saliva facilitates the long-term bonding of *Candida* to the mucosa. In potentially malignant diseases with *Candida* infection superimposition, nitrosamines produced by *Candida* can -directly or with other carcinogenic substances- activate specific proto-oncogenes and induce the development of malignant lesions [26].

Also, in non-secretors, lower IgA levels were reported in both serum and saliva. Therefore, it is likely that specific immune responses are weaker at mucosal surfaces of non-secretors, in comparison with secretors. Lower levels of IgG have also been observed in these individuals [13].

The study of Shahidi Dadras et al., (2016) on the secretor status of patients with oral lichen planus supported the hypothesis that the blood gene antigens on cell surfaces played a role in protecting mucosal surfaces from external pathogens [32].

In conclusion, the significant difference between secretor and non-secretor status in cancerous patients indicates that people with non-secretor status may be more prone to develop head and neck cancer. The presence of these antigens in saliva may have a protective effect.

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