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

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Abstract

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 was 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, researchers 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 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 Halilik 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’ accompanies 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

| Test tube | 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 physiological saline + one drop of an anti-B+ suspension of RBC B type         |
| Test tube 4 | One drop of physiological saline + one drop of an anti-A+ suspension of RBC A 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 ± 14.84 years with head and
neck and 53 controls (26 women and 27 men) with a mean age of 49.13 ± 11.13 years participated. All patients in the case group were 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 sarcomatous 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 3 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.)

| 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 ± 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).

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 Dadars 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.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin. 2015; 65(1):5-29. https://doi.org/10.3322/caac.21254 PMid:25559415

2. Mirzaei M, Hosseini S-A, Ghonecheh M, Soheilipour F, Soltani S, Soheilipour F, et al. Epidemiology and trend of head and neck cancers in Iran. Glob J Health Sci. 2016; 8(1):189. https://doi.org/10.5539/gjhs.v8n1p189 PMid:26234980 PMCID:PMC4803954

3. Ragin C, Modugno F, Gollin S. The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. J Dent Res. 2007; 86(2):104-14. https://doi.org/10.1177/154405910708600202 PMid:17251508
4. Argiris A, Karamouzis MV, Raben D, Ferris RL. Head and neck cancer. The Lancet. 2008; 371(9625):1695-709. https://doi.org/10.1016/S0140-6736(08)60728-X

5. McMahon S, Chen AY. Head and neck cancer. Cancer Metastasis Rev. 2003; 22(1):21-4. https://doi.org/10.1023/A:102293816340 PMid:12716033

6. Bahktiar, S, Jafarian, H, et al. Tobacco and cancer: recent epidemiological evidence. J Natl Cancer Inst. 2004; 96(2):99-106. https://doi.org/10.1093/jnci/dih014

7. Offietta P, Hashibe M. Alcohol and cancer. The Lancet Oncol. 2006; 7(2):149-56. https://doi.org/10.1016/S1470-2045(06)70577-0

8. Dado, M, Pileggi C, Nobile CG, Semino Mayer S. Human papillomavirus and head and neck cancer: a systematic review and meta-analysis. Clin Otolaryngol. 2006; 31(4):259-66. https://doi.org/10.1111/j.1749-4409.2006.01240.x PMid:16911640

9. Pavia M, Pileggi C, Nobile CG, Angelo IL. Association between fruit and vegetable consumption and oral cancer: a meta-analysis of observational studies. The Am J Clin Nutr. 2006; 83(5):1126-34. https://doi.org/10.1093/ajcn/83.5.1126 PMid:16685056

10. Suárez C, Rodrigo JP, Cabanillas R, Shaha AR, Rinaldo A. Tumours of familial origin in the head and neck. Oral Oncol. 2006; 42(10):956-75. https://doi.org/10.1016/j.oraloncology.2006.03.002 PMid:16857415

11. Sturgis EM, Wei Q. Genetic susceptibility—molecular epidemiology of head and neck cancer. Curr Opin Oncol. 2002; 14(3):310-7. https://doi.org/10.1097/00001622-20020500-00010

12. Daniels, G. Human Blood Groups: Introduction, in Human Blood Groups, 3rd edition, Wiley Blackwell, Oxford, UK, 2013. https://doi.org/10.1002/9781118493595.ch1

13. Javaud C, Dupuy F, Maftah A, Julien R, Petit JM. The fucosyltransferase gene family: an amazing summary of the underlying mechanisms of gene evolution. Genetica. 2003; 118(2-3):157-70. https://doi.org/10.1023/A:1024101625214 PMid:12868606

14. Daniels, G. Human Blood Groups: Introduction, in Human Blood Groups, 3rd edition, Wiley-Blackwell, Oxford, UK, 2013. https://doi.org/10.1002/9781118493595.ch1

15. Dayaprasad GK, Venkatesh D. Non-secretor status; a predisposing factor for vaginal candidiasis. Indian J Physiol Pharmacol. 2004; 48:225-9.

16. Thom S, Blackwell C, MacCallum C, Weir D, Brettie R, Kinane D. et al. Non-secretion of blood group antigens and susceptibility to infection by Candida species. FEMS Microbiol Immunol. 1989; 47(6-7):401-5. https://doi.org/10.1111/j.1574-6968.1989.tb02428.x PMid:2631880

17. Salih K, Jouda J, Al-Jaff S, El-Haboby B, Shaker E. Frequency of ABO blood groups and secretor status and their relation with dental decay in a section of students and employees of Al-Mustansiriya University in Iraq. World J Exp Biol. 2015; 3:108-12.

18. Jaffar B, Elhaboby B, Shaker E. Frequency of ABO blood groups and secretor status in patients with Head and Neck Squamous Cell Carcinomas and Related Variables in Al-Havaz City: a 10-Year Retrospective Study. Asian Pac J Cancer Prev. 2017; 18(2):375-379. PMid:28345334 PMCid:PMC5454730

19. Salih K, Jouda J, Al-Jaff S, El-Haboby B, Shaker E. Frequency of ABO blood groups and secretor status and their relation with dental decay in a section of students and employees of Al-Mustansiriya University in Iraq. World J Exp Biol. 2015; 3:108-12.

20. Pradhan A, Chawla T, Samuel K, Pradhan M. The relationship between periodontal disease and blood groups and secretor status. J Periodontal Res. 1971; 6(4):294-300. https://doi.org/10.1111/j.1600-0765.1971.tb00620.x PMid:4272022

21. Lamey PJ, Darwazeh AM, Muirhead J, et al. Chronic hyperplastic candidosis and secretor status. J Oral Pathol Med. 1991; 20:54-67. https://doi.org/10.1111/j.1600-0714.1991.tb00891.x

22. Shin E-S, Chung S-C, Kim Y-K, Lee S-W, Kho H-S. The relationship between oral Candida carriage and the secretor status of blood group antigens in saliva. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003; 96(1):48-53. https://doi.org/10.1016/S1079-2140(03)00160-4

23. Cerovlic V, Juretic M, Balen S, Belusic M, Caser L, Rogic M. Examining the presence of ABO (H) antigens of blood types in the saliva of patients with oral cancer. Coll Antropol. 2008; 32(2):509-12. PMid:18756902

24. Lamey P-J, Douglas P. Secretor status and oral cancer. Br J Oral Maxillofac Surg. 1994; 32(4):214-7. https://doi.org/10.1016/0266-4356(94)90205-4

25. Pai R, Acharya S, Hallikeri K. Assessment of ABO blood grouping and secretor status in the saliva of the patients with oral potentially malignant disorders. J Oral Maxillofac Pathol. 2015; 19(2):164. https://doi.org/10.4103/0973-029X.164527 PMid:26604491 PMCid:PMC4611923

26. Hallikeri K, Udupa R, Guttal K, Naikmasur V. Analysis of salivary secretor status in patients with oral submucous fibrosis: a case-control study. J Invest Clin Dent. 2015; 6(4):261-6. https://doi.org/10.1111/jcld.12100 PMid:24850779

27. Jamil S, Tayyab M, Farooq M. Relationship of secretor status in patients with oral cancers. Annals of KEMU. 2016; 11(4). https://doi.org/10.21649/akemu.v11i14.1113

28. Karpati K, Braunitzer G, Toldi J, et al. Secretor status and ABH antigens expression in a Hungarian population of children and adolescents: an exploratory study. Caries Res. 2014; 48:179–185. https://doi.org/10.1159/000356951 PMid:24480885

29. Campi E, Escovitch L, Valdés V, García Borrás S, Racca L, Racca A, et al. Secretor status and ABH antigens expression in patients with oral lesions. Med Oral Patol Oral Cir Bucal. 2007; 12(6):431-4.

30. Bahktiar S, Toosi P, Dolati F, Bakhshi M. Evaluation of salivary secretor status of blood group antigens in patients with oral lichen planus. Med Princ Pract. 2016; 25(3):266-9. https://doi.org/10.1159/000442291 PMid:26554378 PMCid:PMC5568863

31. Garrett J, Whitlaker J, Nicholson A, Ridway J, Bowman C. Blood-groups and secretor-status in patients with salivary-gland tumours. The Lancet. 1971; 298(7735):1177-9. https://doi.org/10.1016/S0140-6736(71)90490-9

32. Vidas I, Temmer-Vukas B. Examining the secretor status in the saliva of patients with oral pre-cancerous lesions. J Oral Rehabil. 1999; 26(2):177-82. https://doi.org/10.1046/j.1365-2842.1999.00314.x PMid:10080317

33. Shahidi-Dadras M, Golafshan A, Abdollahimajd F. Evaluation of Secretory State in Patients with Oral Lichen Planus: A Case-Control Study. Arch Iran Med. 2016; 19(1):35-8. PMid:26702746