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Original scientific paper

Examining the Presence of ABO(H) Antigens of Blood Types in the Saliva of Patients with Oral Cancer

Robert Cerović[1], Mirna Juretić[1], Sanja Balen[2], Margita Belušić[1], Linda Caser[2] and Mate Rogić[1]

[1] Maxillofacial and Oral Surgery Clinic, University Hospital Center, Rijeka, Croatia
[2] Department of Transfusiology, University Hospital Center, Rijeka, Croatia

A B S T R A C T

Number of researches dealing with the influence of the ABO blood group antigens on the development of the oral cancer have hypothesized that people who do not secrete these substances in the saliva are more prone to suffer from this disease. The objective of this research is to examine this hypothesis. In total 114 subjects were examined, half of which suffered from oral cancer, while the other half was the healthy control group. All examinees were subjected to clinical examinations and the experimental group to pathohistological examination. An analysis of the secretor status was carried out using the Wiener agglutination test. The experimental group consisted of 78,95% of secretors, while the control group consisted of 82,46% of secretors. This difference is not statistically significant. The starting hypothesis that non-secretors are more prone to the development of oral cancer was not confirmed.

Key words: *secretor status, ABO antigens, oral cancer*

Introduction

Oral cancer has a significant place in the epidemiology of malignant diseases. Head and neck squamous cell carcinoma was the eight leading cause of cancer death worldwide in 2000[1].

The risk factors for oral cancer have been studied in detail. Alcohol, tobacco smoking and nutritional status are well known factors associated with the increased risk of oral cancer. Other possible factors in the development of oral cancer, such as Human Papillomavirus and ABO antigens, are also being researched[1,2]. Existing researches link these factors with the development of cancer, but the results are different and still only hypothesis which have to be proven. A possible risk marker for the susceptibility to oral cancer is patients' secretor status. Some people posses the ability to secrete blood group substances in the saliva and they are referred to as »secretors«, whereas others who lack such an ability are referred to as »non-secretors«. It has been demonstrated in a number of earlier studies that the patient's secretor status may probably be a factor influencing the development of systemic oral disease [3].

Immunohystochemical studies of oral squamous cell carcinomas have shown loss of expression of A or B antigens in more than 80% of cases. Studies of potentially malignant lesion have shown loss of A/B antigen in most lesions with epithelial dysplasia, and in the half of the lesions clinically classified as *leukoplakia* but without histological evidence of epithelial dysplasia[4,5].

Lamey et al. (1994) 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[2]. Few years later Vidas et al. (1999) examined the influence of the secretor status on certain precancerous lesions in oral cavity and stated that an inability to secrete blood group antigens in saliva could be regarded as a risk factor in the development of oral cancer[3].

The goal of this research was to examine the hypothesis regarding the influence of secretor status on the development of oral cancer in a selected Croatian-European population.

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Materials and Methods

57 patients with diagnosed oral carcinoma were examined, along with the same number of people with no pathological changes in the mouth in the control group. The experimental group consisted of 47 men ad 10 women. The average age of examinees was 62. The control group consisted of the same number of men and women as the experimental group, but the average age was 60. A biopsy and histopathological analysis has been done on all the patients, and they have all had a planocellular carcinoma diagnosed. Stages of diseases varied a lot, depending on when they had contacted their medic. All the patients had primary tumor and none had been previously treated for the same disease.

Data were examined with a $\chi 2$ test.

Establishing the secretor status in the saliva[3]

1 ml of no stimulated saliva has been collected from each patient, by a negative pressure suck-pump, into a sterile glass jar, and afterwards 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. Applying the Wiener agglutination test the secretor status was analyzed. Test serum used in this experiment, was diluted in a salted physiological solution in proportion 1:10, the same proportion that the saliva was diluted in.

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

I 1 drop of saliva + 1 drop of anti-B serum

II 1 drop of saliva + 1 drop of anti-A serum

III 1 drop of physiological solution + 1 drop of anti-B serum

IV 1 drop of physiological solution + 1 drop of anti-A serum

After 10 or more minutes at room temperature, 1 drop of 2–3% of suspension A_erythrocytes was added into sterile tube II and IV, and 1 drop of suspension B erythrocytes into tube I and III. All the test tubes were shook, and left on room temperature. After one hour the results were ready for reading.

Test-tubes III and IV were control and agglutination occurred in them. Agglutination_in tube I is a result of the presence of substance A2 in a saliva, i.e. of sector A, while the agglutination in tube II is a proof of B sector. The absence of agglutination in tubes I and II designated AB secretor, and at the same time agglutination in tubes I and II has proven that the person is non-secretor.

Results

Most of our examinees in, both experimental and in control group are secretors. The_secretor status of patients with oral carcinoma is shown in figure 1, and the status of the healthy, control group, is shown in figure 2.

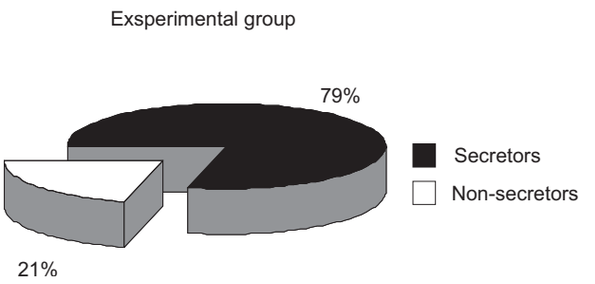

Fig. 1. Secretor status of 57 examinees in the experimental group (79%) which consisted of 45 secretors and 12 non-secretors (21%).

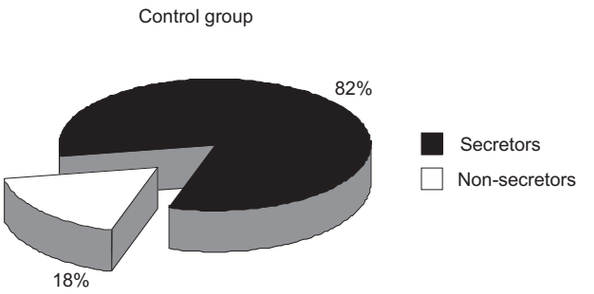

Fig. 2. Secretor status of 57 examinees in the control group which consisted of 47 secretors (82%) and 10 non-secretors (18%).

TABLE 1
RELATION OF SECRETOR STATUS REGARDING SEX AND AGE OF EXAMINEES

	Experimental group		Control group	
	Secretors	Non-secretors	Secretors	Non-secretors
Men	39	8	40	7
Women	6	4	7	3
Total	45	12	47	10

In the experimental group 45 examinees out of 57, or 78.95%, are secretors while 12 patients, or 21.02% do not have blood group antigen in saliva.

47 people in the control group are secretors, or 82.46% and 10 people are non-secretors, or 17.54%. The difference between control and experimental groups does not have statistical significance ($p>0.05$).

Table 1 shows test results of the secretor status regarding sex. We can see that out of total of 47 men in the experimental group, 8 (17%) are non-secretors, and 39 are secretors (83%). In the control group 40 men are secretors (85.1%), and 7 are non-secretors (14.9%) Out of 10 women in the experimental group 6 of them (60%) are secretors, and 4 women are non-secretors (40%). 7 women in the control group are secretors (70%) and 3 are non-secretors (30%).The differences between the control and the experimental group are not statistically significant ($p>0.05$).

Discussion

A number of studies show the relation between the expression of A or B blood group antigens and malignant tumors[4,6–8]. Experimental studies of rat colon carcinoma cells indicate that cells with A expressions are *tumorogenic* but cannot be compared with human carcinogenesis because the expression of blood group antigen is opposite that seen in the human[4,8]. Studies with human colon carcinoma cell lines have shown that loss of expression of AB *glycosyltransferase* can enhance malignancy of the cell lines[4,9]. Studies of bladder cancer indicate that loss of A/B expression involves the deletion of large chromosomal region including the ABO locus at 9q34 1–2, which may contain one or more tumor suppressor genes[4,7]. In the normal oral cavity, keratinized epithelium in the palate or gingiva shows little or no expression of A or B blood group antigen. Since a change from a non-keratinized to a keratinized differentiation pattern is a characteristic of many oral carcinomas and potentially malignant lesions, the lack of expression of blood group in such lesions could be due to a change in differentiation pattern of the epithelium[4]. However, it has been demonstrated that half of the *leukoplakias* that developed in the buccal mucosa show expression of A antigen, even tough they histologicaly appear as keratinized lesions[4,5]. These findings indicate that loss of antigen is not necessarily associated with *hyperkeratinization* or even with oral cancer.

Lamey et al. (1991) investigated the secretor status in saliva of a group of patients with chronic *hyperplastic candidiasis*, and compared with a corresponding control group of healthy examinees. They discovered that 68% of those having the disease were non-secretors, whereas the percentage of non-secretors in the control group was statistically significantly lower. *Candidal leukoplakia* was considered as belonging to precancerous lesions, therefore, a hypothesis that the non-secretor status may have an impact in the pathogenesis of oral cancer in non-secretors should be considered[3,10].

The motive for this research was the article written by a group of authors – Vidas et al. (1999), who examined the influence of secretor status on certain precancerous lesion in oral cavity[3]. What is stated in the article is the notion that non-secretors have a more intensive disease, with higher probability of gaining epithelial dysplasia, what leads us to a conclusion that non-secretors might be more prone to the development of oral carcinoma. Even though such a research was already conducted (Lamey et al. 1994)[2], we wanted to examine the patients from the same population (European) that was examined in the research by Vidas et al., because the research by Lamey et al. was conducted on the Sri Lankan population.

The results did not show a significant difference between the secretor and non-secretor status in the research and the control group (17% non-secretors in the research group and 15% in the control group). Similar results can be found in Lamey et al. research of the secretor status in the saliva of oral carcinoma patients[2].

Since the Vidas et al. experiment included mostly female population, and we could not have chosen such a sample, because most of oral carcinoma patients are men, we tested the relation between the secretor status and the sex of our examinees. Even here we could not find significant differences between the control and the research group.

Authors of previously mentioned article tested a couple of changes that can be considered as pre-cancerous lesions, *leukoplakia* and *lichen ruber planus* and *erosivus*. However, only a few of those changes have strong epithelial dysplasia and those that do contain such a pathohistological component, rarely alter into malign. Precancerous lesion that has a more significant malign alteration is *erythroplakia*[11,12], but Vidas et al. research did not include a single examinee with such an alteration in oral cavity. Authors state that there is significantly larger percentage of non-secretors found in patients that have precancerous lesion with strong epithelial dysplasia, therefore more non-secretors can be found among patients with oral carcinoma[3]. The results of this study do not confirm this hypothesis.

Conclusion

Though existing laboratory and clinic researches do not show the correlation between ABO blood group antigens and the development of oral carcinoma, this is not clinically confirmed. A few existing clinic researches have been conducted on a small number of examinees and results vary a lot. Based on this brief research we can not state that there is a correlation between the presence of ABO antigens in the saliva and the development of oral carcinoma. The starting hypothesis that non- secretors are more prone to development of oral carcinoma is not confirmed.

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R. Cerović

Maxillofacial and Oral Surgery Department, Clinical Hospital Center Tome Strižića 3, 51000 Rijeka, Croatia
e-mail: robert.cerovic@ri.t-com.hr

ISPITIVANJE PRISUTNOSTI ABO(H) ANTIGENA KRVNIH GRUPA U SLINI BOLESNIKA OBOLJELIH OD ORALNOG KARCINOMA

S A Ž E T A K

U malobrojnim, do sada objavljenim istraživanjima o uticaju ABO antigena krvnih grupa na razvoj oralnog karcinoma, postavljena je hipoteza da bi osobe koje nemaju sposobnost lučenja tih supstanci u slini (nesekretori), mogle biti sklonije obolijevanju od te bolesti. Cilj ovog istraživanja bio je ispitati navedenu hipotezu. Ukupno je ispitano 114 ispitanika, od kojih je polovica bolovala od oralnog karcinoma, dok su drugu, kontrolnu grupu sačinjavali zdravi ispitanici. Svi su ispitanici klinički pregledani, a eksperimentalnoj grupi učinjena je patohistološka dijagnoza. Ispitivanje sekretornog statusa učinjeno je pomoću Wienner-ovog testa aglutinacije. U eksperimentalnoj grupi bilo je 78,95% sekretora, dok je u kontrolnoj grupi bilo 82,46% sekretora. Ta razlika nije statistički značajna. Početna hipoteza da su nesekretori skloniji obolijevanju od oralnog carcinoma nije potvrđena.