Analysis of the FUT2 gene and Secretor status in patients with oral lesions

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RESUMEN

Los mecanismos de la expresión aberrante de los antígenos de grupo sanguíneo no son claros en todos los casos. El objetivo de este trabajo fue investigar los antígenos de grupo sanguíneos Lewis y el estado secretor utilizando métodos bioquímicos y de biología molecular de pacientes con lesiones orales. Estudiamos 148 sujetos, la mitad de los cuales sufrían de lesiones orales (benignas, pre-cancerosas y cancerosas), mientras que la otra mitad fue el grupo control. Se investigó los fenotipos Lewis en muestras de sangre fresca a través de técnicas de hemaglutinación y el carácter secretor de los mismos a partir de muestras de salivas por inhibición de la hemaglutinación. Se analizó el polimorfismo del gen FUT2 (que codifica la α(1,2) fucosiltransferasa, que regula la expresión de los antígenos ABH en el tracto gastrointestinal y en las secreciones) por ASO-PCR (oligonucleótido alelo específico, “allele specific oligonucleotide– polymerase chain reaction”) con cebadores específicos para el alelo G428 y el alelo de tipo salvaje del gen FUT2. Se encontró un mayor grado de malignidad de las enfermedades orales en el grupo no secretor (OR = 3.44). El 51% de los pacientes con lesiones precancerosas y cancerosas fueron no secretores con fenotipo Le (a + b -), en contraste con la población control. Se observó una asociación marginal entre el estado secretor y estas lesiones.

El carácter no secretor y la ausencia del alelo salvaje FUT2 podrían ser considerados marcaproyces de riesgo en aquellos pacientes que presentan lesiones orales.

PALABRAS CLAVE: Gen FUT2 / Estado secretor / Pre cancer / Cancer / Oral.

ABSTRACT

The mechanisms of aberrant expression of blood-group antigens are not clear in all cases. The aim of this work was to investigate Lewis blood type antigens and secretor status using biochemical and molecular biological methods in patients with oral lesions. We studied 148 subjects, half of whom suffered from oral lesions (benign, pre-cancerous and cancerous), while the other half were the healthy control group. We also investigated the Lewis phenotypes of fresh blood samples with a hemagglutination method. We analyzed polymorphisms of the FUT2 gene by ASO-PCR (allele specific oligonucleotide– polymerase chain reaction) with specific primers for the G428 allele and the wild type allele of FUT2 gene. The FUT2 gene encodes the α(1,2) fucosyltransferase (Se enzyme) that regulates expression of ABH antigens in the gastrointestinal tract and secretions. We found a higher intensity of oral disease in the non-secretor group (OR = 3.44). Fifty one % of the patients with oral pre-cancerous and cancerous lesions were non-secretors (Le a+ b-), in contrast with the healthy population. We observed a marginal association between secretor status and these lesions.

Our study suggests that the lack of wild type FUT2 gene and a non-secretor status appear to be an associated risk marker for the development of oral cancer in patients with oral lesions.

KEY WORDS: FUT2 gene / Secretor Status / Oral pre cancer / Oral cancer.
INTRODUCTION
The Lewis histo-blood group antigens Lewis a (Lea) and Lewis b (Leb) are carbohydrate structures that form epitopes on glycolipids and glycoproteins. Two independent genes determine the Lewis phenotype; the Lewis gene (Le and le), and the secretor gene (Se and se). Conventional Lewis blood grouping is difficult (e.g., in cancer patients and pregnant women) because of the presence of nongenuine Lewis-negative individuals. The secretor status in Lewis-negative individuals is currently determined by a labor-intensive hemagglutination inhibition technique that uses heat-inactivated saliva. In Lewis positive individuals, the secretor status is deduced from the Lewis phenotype: i.e.: Le(a-b+) individuals are secretors, and Le(a+b-) individuals are non-secretors.

The H antigen, which is a precursor of A and B antigens, is synthesized by α(1,2) fucosyltransferase. It has been demonstrated that two distinct α(1,2) fucosyltransferases are present in human tissues. One is the H gene (FUT1)-encoded α(1,2) fucosyltransferase (H enzyme) that regulates expression of ABH antigens in erythrocytes, and the other is the Secretor gene (FUT2)-encoded α(1,2) fucosyltransferase (Se enzyme) that regulates expression of ABH antigens in the gastrointestinal tract and secretions. Secretors, who have ABH antigens in saliva, have at least one functional Se allele, and non-secretors, who fail to express ABH antigens in their saliva, are homozygous for the nonfunctional se allele.

The FUT2 gene has a significant polymorphism with typical ethnic specificity. The nonsense mutation 428G→A (Trp143→stop) is characteristic for the dominating nonsecretor allele (se428) in Europeans and appears in about 20% of the Caucasian population.

Since most human cancers originate from epithelial cells, changes in blood group antigens are an important topic in human tumor immunology. Glycolipids constitute an essential part of blood group antigens present at the cell surface membranes. In human tumors, blood group antigens change in the same general direction as other glycosphingolipids do in tumors. Tumor progression is often associated with altered glycosylation of the cell-surface proteins and lipids. The peripheral part of these cell-surface glycoconjugates often carries carbohydrate structures related to the ABO and Lewis blood-group antigens. The expression of histo-blood-group antigens in normal human tissues is dependent on the type of differentiation of the epithelium. In most human carcinomas, including oral carcinoma, a significant event is decreased expression of histo-blood-group antigens A and B. The mechanisms of aberrant expression of blood-group antigens are not clear in all cases. It has been demonstrated in a number of earlier studies on the etiology and pathogenesis of certain diseases that the patients’ secretor status and Lewis blood group antigens may probably be a factor influencing the development of systemic diseases.

In the present study, we analyzed the FUT2 gene and Se status in patients with oral lesions (benign, pre-cancerous and cancerous lesions).

MATERIALS AND METHODS
In total 148 subjects were examined, half of whom suffered from oral lesions (benign, pre-cancerous and cancerous), while the other half were the healthy control group. All of them were subjected to clinical oral examinations and standard evaluation tests in order to establish the secretor status of their saliva (agglutination inhibition technique). In the group of patients with oral benign, pre-cancerous and cancerous lesions (experimental group), a pathohistological examination of the oral mucosa was performed.

Appropriate informed consent was obtained from all subjects and all procedures were performed according to the ethical standards established by the University of Rosario.

Serological studies
Saline erythrocyte suspensions were used for serological studies.

The Lewis phenotypes of fresh blood samples were determined by a hemagglutination method, using anti-Lea and anti-Leb monoclonal antibodies.

In order to establish the secretor status we analyzed their saliva by the agglutination inhibition technique.

Inhibition test for Secretor Status:
Two or 3 ml of saliva were collected into wide mouthed tubes. In order to eliminate the mucine protein they were treated with thermal shocks. They were then centrifuged and the supernatants were transferred to clean test tubes. In order to macroscopically inspect for agglutination.

To 1 drop of appropriately diluted blood grouping reagent (anti-A, anti-B, or Ulex europaeus lectin) we added 1 drop of the patient’s saliva. After incubation for 10 minutes at room temperature, we added 2 drops of 2% to 5% saline suspension of washed indicator red cells. Then, the tube was incubated for further 30 minutes and centrifuged in order to macroscopically inspect for agglutination.

Agglutination of indicator cells by antibody in tubes containing saliva indicates that the saliva does not contain the corresponding antigen (non-secretor status). Failure of known antibody to agglutinate indicator cells after incubation
with saliva indicates that the saliva contains the corresponding antigen (secretor status).

**Molecular studies**

**DNA isolation**

Genomic DNA was isolated from saliva samples with a modified salting-out procedure. The DNA concentration was measured spectrophotometrically at 260 nm and diluted in sterile water to a concentration of 100 ng per μl (18).

**G428A polymorphism**

The DNA samples were analyzed by ASO-PCR (Allele Specific Oligonucleotide – Polymerase Chain Reaction) with specific primers (Operon Lab) for the G428 allele and the wild type allele of FUT2 gene (Table I). A fragment of 132 bp was amplified as described by Henry et al. (18), except for modifications of the annealing temperature according to the Tm of the primers.

**Statistical Analysis**

The categorical data were examined with a χ2 test, and the ORs were estimated using an unconditional logistic model.

**RESULTS**

79.5 % of the healthy individuals studied possessed the Se gene (FUT2) that governs the secretion of water-soluble ABH antigens into saliva. These secreted antigens can be demonstrated in saliva by agglutination inhibition tests with ABH antisera and molecular biology through analysis of the FUT2 gene. 51% (n=21) of the patients with oral pre-cancerous and cancerous lesions were non-secretors (Le a+ b-), OR = 2.44; II 95% (0.7836; 7.5534) (p= 0.1196) in contrast with the healthy population (Table II). We observed a marginal association between the secretor status and these lesions.

The molecular analysis showed that 28.38 % of the patients were homozygous for the G428A mutation (the mutation present in the 2 alleles), the other patients were homozygous for the secretor status (none of them presented the allele G428A), or heterozygous secretor (1 allele presented with the mutation G428A).

We also found a higher intensity of oral disease in the non-secretor group, OR = 3.44; CI 95% (1.0682; 11.0729) (p= 0.0346), and the occurrence of epithelial dysplasia was found exclusively in the non-secretor group.

**DISCUSSION**

Lewis antigens are genetically, immunochemically, and biochemically closely related to the A, B, H blood group antigens. They are present in saliva and other secretions, as well as in the blood plasma (2,3). The determination of Lewis and secretor histo-blood group status has until now relied on immunological reactivity with Lewis carbohydrate antigens on erythrocytes, complemented with the determination of the presence or absence of ABH antigen in the saliva of Lewis-negative individuals by the hemagglutination inhibition test (17).

People who do not secrete their blood type antigen into their secretions are termed “non-secretors”. About 20% of the Caucasian population is non-secretor (8). Several correlations to disease have been linked to non-secretor status. Generally speaking, being non-secretor results in several disadvantages with regard to metabolism and immune function (4,8). The gene interaction between Lewis and secretor genes accounts for a classification into three Lewis blood types and explains the close association between Lewis blood types and saliva ABH secretor types: Erythrocyte individuals (20%) are saliva ABH non-secretors (9). Our results have demonstrated that

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**TABLE I.** Sequence of primers for the analysis of the G428A mutation

<table>
<thead>
<tr>
<th>Primers</th>
<th>Tm</th>
<th>Sequence</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>FUT2-Se-428-s</td>
<td>68.8 ºC</td>
<td>5'-CCGGGTACCCCTGCTCGTG-3'</td>
<td>Se (Forward)</td>
</tr>
<tr>
<td>FUT2-se-428-f</td>
<td>66.6 ºC</td>
<td>5'-ACCCTACCCCTGCTCGTA-3'</td>
<td>se (Forward)</td>
</tr>
<tr>
<td>FUT2-all-523-as</td>
<td>66.7 ºC</td>
<td>5'-CCGCTCCCGTTACCTCGT-3'</td>
<td>(Reverse)</td>
</tr>
</tbody>
</table>

**TABLA II.** Secretor status in patients with oral lesions

<table>
<thead>
<tr>
<th>Secretor Status</th>
<th>Benign Lesions (mucosceles, papiloma, etc)</th>
<th>Pre-cancerous and Cancerous Lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le(a-b+)</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Le(a+b-)</td>
<td>12</td>
<td>21</td>
</tr>
</tbody>
</table>

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most individuals within the healthy group were secretors (79.5 ± 5), and that there was a significant difference between secretors and non-secretors in the experimental group. We also found a higher intensity of oral disease in the non-secretor group, and the occurrence of epithelial dysplasia was mainly found in this group. We also observed that the red cell Lewis antigen reactivity does appear to be associated with the secretor status in the saliva, a conclusion supported by the observation that some individuals with Le(a-b+) red cells show reactivity of ABH antigens in their secretions and they have the FUT2 gene.

This study evaluated the association between oral lesions and polymorphisms of the Se genes and secretor status. We found that oral pre-cancerous and cancerous lesions were increased among individuals with non-secretor status and nonsense mutation 428G→A (Trp143→stop). We also demonstrated that the Le a+b+ antigen expression was present in the population showing greater risk.

The studies of patients with pre malignant and malignant oral lesions, in which non-secretor status predominates, show that this status appears to be an associated risk marker for the development for oral cancer.

In summary, our findings demonstrate that the Se genotypes affect the risk of developing malignant oral disease defined by the Secretor status. Further follow-up studies are required to clarify the role of predictive markers of risk in precursor lesions of oral cancer.

DISCLOSURES

The authors declare no financial conflict of interest.

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