Secretor status, *Fut 2* gene and ABH antigens in urogenital tumors

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Abstract—

Background: *Blood group antigens are polymorphic, inherited structures located on the surface of the red blood cell. The mechanisms of aberrant expression of blood-group antigens are not clear in all cases.*

Aim: *to evaluate the expression of FUT2 gene in saliva and histo ABH antigens of patients with urogenital tumours in order to determine whether this factor could be a marker risk of urogenital cancer.*

Methods: *128 subjects were examined, half of whom suffered from urogenital lesions, while the other half was the healthy control group. All were subjected to clinical examinations and standard evaluation tests in order to establish the secretor status of their saliva. For the molecular studies the saliva samples were subjected to thermal shock, centrifuged and the genomic DNA was extracted by an enzymatic digestion method. The DNA samples were analyzed by ASO-PCR with specific primers for the G428A allele and for the wild type allele of the FUT2 gene. To reveal A, B and H antigens in tissue sections of the patients we used a modified specific red cell adherence technique.*

Results: *We found a higher intensity of disease in the non-secretor group (OR = 2.44) and the occurrence of epithelial dysplasia was found exclusively in this group. The 51.2% of the patients with urogenital cancerous was non secretors, in contrast with the healthy population (22.1%). In the tissues analyzed the test showed slightly positive results on atypical areas, and there was a complete antigen deletion in areas histological affected by neoplasia. Further it is suggested that areas of blood group isoantigen negative epithelium showing atypia, or in some instances near normal histology, may give rise to relatively low grade carcinomas.*

Conclusions: *Considering these results we suggest the use of this method to monitor probable preneoplastic lesions in risk population, especially in those with no secretor status.*

Keywords— Fut 2 gene, ABH antigens, urogenital, tumors.

I. INTRODUCTION

Cancer incidence in humans has gradually increased over the last century. Surgical, radio, chemotherapeutic and biological treatments have experienced important advances, with concomitant reduction in the morbidity associated with the radical surgical practices of the past [1,2].

The diagnosis of prostatic lesions especially the prostatic adenocarcinoma is challenging and surprising to the pathologist. As these carcinomas have various histomorphological patterns of presentation with minimal cytological and architectural atypia in limited tissue fragment like needle biopsy [3,4]. The histomorphological variants in prostatic tumor like atrophic, foamy gland, pseudohyperplastic and certain subtypes of ductal adenocarcinoma represent the most common causes of under-diagnosed cancer [5].

Blood group antigens are polymorphic, inherited structures located on the red blood cell surface. The ABO blood group antigens are among the well-known fucosylated glycans. The expression of them is regulated by several glycosyltransferases that add monosaccharides to a precursor molecule in a sequential fashion [6,7]. The α(l,2)fucosyltransferase that forms the H antigen, an essential precursor of the A and B antigens, plays a regulatory role in the tissue expression of the ABO antigens [7].

The expression and secretion of ABO antigens in epithelial cells are controlled by secretor type α(l,2)fucosyltransferase activity, known as the Secretor (Se) transferase (*FUT2* gene product). Several different polymorphisms are known in the *FUT2* gene, some called as silent mutations, while others as to non-functional enzymes [7,8].

Homozygous individuals with non-functional enzymes are termed non-secretors (se_/_). About 20% of individuals are non secretors who fail to express the ABO antigen in saliva. On the other hand, heterozygous individuals carrying one functional allele, have secretion similar to the wild-type. These are termed secretors (Se) [9].

The functional significance for ABO antigen expression on erythrocytes has not been defined, but ABO-related structures may play a role in other systems. Alterations in the expression of fucosylated oligosaccharides have also been observed in several pathological processes, including cancer and atherosclerosis [10,11].

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 [12,13].

The expression of histo-blood group antigens in normal human tissues is dependent on the type of differentiation of the epithelium. The mechanisms of aberrant expression of blood-group antigens are not clear in all cases [14]. It has been demonstrated 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 probably be a factor influencing the development of systemic oral diseases [15]. This likelihood has prompted the present study, to examine the differences in secretor status in patients with urogenital tumors; in relation to the ABH antigens expression in fixed tissue sections of these patients.

II. MATERIALS AND METHODS

The patients analyzed in this study presented to the Urology Department of the Medicine Faculty of the National University of Rosario. Recruitment was made by consecutive sampling for a period of 20months.

From a total of 128 subjects examined, half suffered from urogenital tumors (bladder, prostatic and kidney) and benign lesions (benign prostatic hyperplasia) while the other half was the healthy control group. All were subjected to clinical examinations and standard evaluation tests in order to establish the secretor status of their saliva. In the group of patients with cancerous lesions (experimental group), a histopathological examination was performed.

All subjects gave informed consent to participate in the study, and the protocol was approved by the Ethic Committee of the School of Biochemestry Sciences of Rosario, Argentina, according to the principles of the Declaration of Helsinki.***Serological studies***

Saline erythrocyte suspensions or saliva were used for serological studies. The Lewis phenotypes of fresh blood samples were determined by a hemagglutination method [16] using anti-Le^a and anti-Le^b monoclonal antibodies.

2.1 Inhibition test for Secretor Status [16]:

2 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 centrifuged and the supernatant were transferred to a clean test tube and placed in boiling water bath for 10 minutes to inactivate salivary enzymes. To 1 drop of appropriately diluted blood grouping reagent (anti-A, anti-B or ulex europeaus) we added 1 drop of patient's saliva. We incubated 10 minutes at room temperature and then we added 2 drops of 2% to 5% saline suspension of washed indicator red cells. Then, the tube was incubated 30 minutes and centrifuged in order to inspect cell button macroscopically 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, se). Failure of known antibody to agglutinate indicator cells after incubation with saliva indicates that the saliva contains the corresponding antigen (secretor status, Se).

III. MOLECULAR STUDIES [17]

3.1 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.

3.2 G428A polymorphism

The DNA samples were analyzed by ASO-PCR (allele spe-cific oligonucleotid–polymerase chain reaction) with specific primers (Operon Lab) for G428A allele and the wild type allele of *FUT2* gene (Table 1). A fragment of 132 bp was amplified as described by Henry et al. except for modifications of the annealing temperature according to the Tm of the primers.

TABLE 1
SEQUENCE OF PRIMERS FOR THE ANALYSIS OF THE G428A MUTATION

Primers	Tm	Sequence	Specificity
FUT2-Se-428-s	68,8 ºC	5'-CCGGTACCCCTGCTCGTG-3'	Se (directo)
FUT2-se-428-f	66,6 ºC	5'-ACCGGTACCCCTGCTCGTA-3'	se (directo)
FUT2-all-523-as	66,7 ºC	5'-CCGGCTCCCGTTCACCTG-3'	No específico (reverso)

IV. SPECIFIC RED CELL ADHERENCE TEST [18]

Specific red cell adherence test was performed on paraffin embedded sections to detect the intensity of isoantigens A, B and H (O) on the epithelial cell surface by a three layer sandwich technique.

4.1 Tissue preparation

All biopsies were fixed in 4% buffered formaldehyde, paraffin embedded, sectioned at 4 μm, and stained with hematoxilyn and eosin. Sections (4 μm) from the tumor biopsies were placed on gelatine-coated slides. Sections were deparaffinized in xylene and brought to water through graded ethanol (100%).

4.2 Reagents Used

- Commercially available antisera, Anti A, Anti B, and AntiAB, from span diagnostic limited and lectin ulex europeaus (Anti H).
- Tris Buffer saline 0.05 M, pH 7.4.
- 2-5% Red Blood Cell's (RBC) suspension.

V. PROCEDURE IN BRIEF

Slides of 4-5 micron section were deparaffinized and brought to water, immersed in tris buffered saline 0.05 M (pH 7.4) for 30 minutes, covered with isologous antisera according to patients' blood group and incubated for one hour for A, B and O group in a most chamber at room temperature. The slides were then dipped in tris buffered saline for three changes with occasional strings to remove the unreacted antisera. Few drops of 2-5% isologous indicator RBC's suspension were added to the sections and incubated for 20 minutes in group A or B and one hour for group O. The slides were inverted over a support in a petri dish containing Tris buffered saline such that the undersurface of the slide just touched the solution and kept for five minutes to settle down unreacted RBCs. The slides were observed under low power and photographed immediately.

5.1 Controls

Normal tissues containing blood group antigens, endothelium of blood vessels and RBCs acted as inbuilt positive controls and adipose tissues acted as inbuilt negative controls.

5.2 Interpretation

In the present study the isoantigenicity of epithelium was graded according to degree of adherence of indicator RBCs as strongly positive adherence (++++) to negative adherence (-), Levels intermediate were determined as 25% of adherence +, 50% of adherence ++ and 75% of adherence +++.

VI. STATISTICAL ANALYSIS

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

VII. RESULTS

The 77.9 % of the healthy individuals studied posses the *Se* gene that governs the secretion of water-soluble ABH antigens into saliva (secretor status). These secreted antigens can be demonstrated in saliva by agglutination inhibition tests with ABH antisera. The 51.2% (n=20) of the patients with urogenital cancerous was non secretors (se) (Le a+ b-), RO = 2.44; IC 95% (0.7836 ; 7.5534) (p=0.1196) in contrast with the healthy population (22.1%) (Table 2). The molecular analysis also showed that 51.2% of patients was 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). The 34.8% (n=10) of the patients with benign prostatic hyperplasia was non secretors. We observed a marginal association between secretor status (Se) and these lesions.

The immunoadherence reaction to tissue sections using antibodies and red blood cells showed a loss of A, B or H antigens related to the stage of tumor. We found a higher intensity of the disease in the non secretor group (se), RO = 3.44; IC95% (1.0682; 11.0729) (p= 0.0346), and the occurrence of epithelial dysplasia was found exclusively in these group. A loss of ABH reactivity within the most invasive sites of the tumors correlated significantly with the stage of tumor development and histological grade of malignancy (Fig 1, 2).In the tissue sections studied, the endothelium of blood vessels was reactive with the erythrocytes (positive control), and adipose tissues did not react with the red blood cells (negative controls).

TABLE 2
SECRETOR STATUS IN PATIENTS WITH ORAL LESIONS

	BENIGN LESIONS	CANCEROUS
Secretor Status Le(a-b+)	19	19
Non Secretor Status Le(a+b-)	10	20

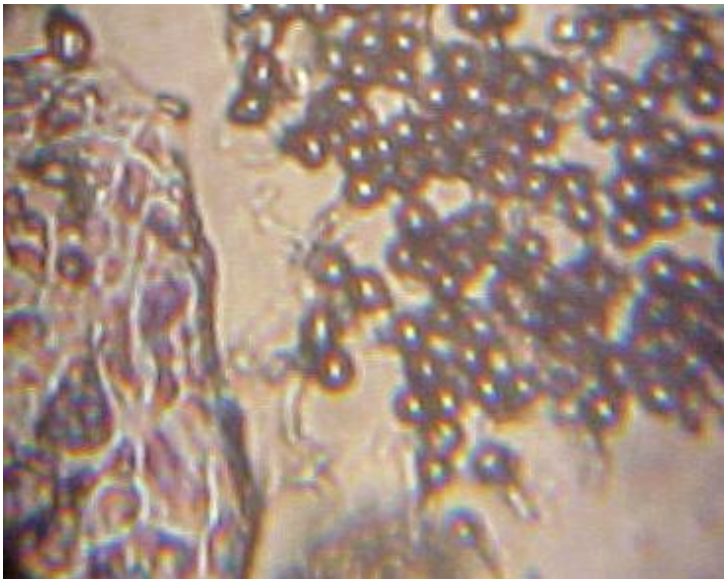

FIG. 1. CANCEROUS LESION: NON INMUNOADHERENCE OF RED BLOOD CELLS

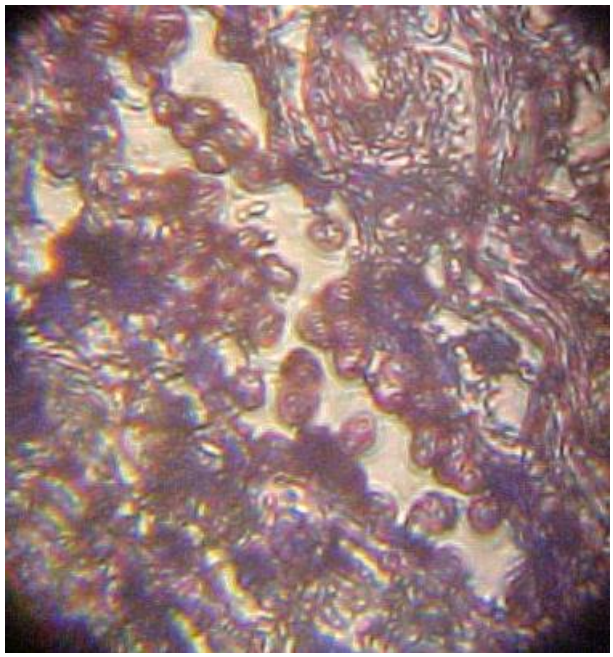

FIG. 2. BENIGN LESION – BENIGN PROSTATIC HYPERPLASIA: INMUNOADHERENCE OF RED BLOOD CELLS TO THE TISSUE

VIII. DISCUSSION

Blood-group antigens can be present on key receptors controlling cell proliferation, adhesion, and motility, such as epidermal growth factor receptor, integrins, cadherins, and CD44. The expression patterns of these various receptors differ according to the type of normal epithelium and the type of cancer, and therefore the role of ABH antigens in the biology of human cancers may also vary. In routine diagnostic histopathology, classification of tumor type is based on the histologic appearance of the most differentiated parts of the tumor [19,20]. The prognosis of the tumor, on the other hand, is based partly on properties within the less differentiated parts. In most cases, the degree of differentiation is determined by cellular and tissue morphology and by the ability of the cells to synthesize certain specific products such as keratin and mucins. In tumors, changes in glycosylation are found in both glycolipids and glycoproteins. Most studies have dealt with alteration of carbohydrates at the cell surface [21.22].

Lewis antigens are genetically, immunochemically, and biochemically closely related to the A, B, and H blood group antigens. They are present in saliva and other secretions, as well as in the blood plasma. 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. 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. Several correlations to disease have been linked to non-secretor status. [23]

The results obtained in this work, have demonstrated that the large majority of the people examined in the healthy group were Se and there were significant difference between secretors and non-secretors in the experimental group. We also found a higher intensity of urogenital disease in this group, and the occurrence of epithelial dysplasia was most found in this group. The studies of patients with malignant lesions, in which non-secretor status predominates (Le a+ b-), appear to be an associated risk marker for the development for urogenital cancer.

We used the loss of the expression of ABH antigens as a marker of differentiation. As the expression of these antigens can be detected by monoclonal antibodies, they are a better objective marker of differentiation than the more commonly used subjective histological assessment. The presence or absence of blood group antigens has been used to predict the clinical course of patients with superficial transitional cell carcinoma of the bladder [24]. The red-cell adherence test has been the most widely accepted method of antigen determination, but this technique has inherent weaknesses. Recently, the immunoperoxidase assay has been used to detect antigens on tumor cells. We compared patients using the red-cell adherence and immunoperoxidase methods on adjacent micro cut. The red-cell adherence and immunoperoxidase methods performed similarly (89%) when assessing the presence or absence of antigen [25].

We observed a reduction or complete deletion of A, B or H antigen expression in sections of tissues of patients with carcinomas. Disappearance of the antigens is ascribed to the absence of A or B transferase gene expression. These findings are consistent with several studies showing that loss of A and B antigen expression is associated with increased tumourigenecity in syngeneic animals.

In summary, our results indicate that at the same time as the morphological changes that occur during the process of carcinogenesis, another series of events occurs. Further follow-up studies are required to clarify the role of predictive markers of risk in precursor lesions of urogenital cancer.

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