DOCTORAL THESIS

SUMMARY

CLINICO-EPIDEMIOLOGICAL STUDY OF URINARY BLADDER TRANSITIONAL CARCINOMA CORRELATED WITH MARKERS INVOLVED IN CELL CYCLE REGULATION

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INTRODUCTION

Urothelial carcinomas are a common group of cancers in oncological urologic pathology, also representing the most common invasive cancer of the urinary tract. Lately there has been an increased incidence of urothelial neoplasia due to exposure to a wide range of potentially carcinogenic substances. Studies of involved factors led to the concept of existence of so-called malignization terrain, which claims that individual genetic predisposition and chronic exposure to carcinogens act synergically leading to the appearance of urothelial carcinomas of the bladder.

This paper aims to address an issue, and therefore a health problem of great interest and importance to both the high and growing incidence and the impact it has on health in general, trying to find appropriate solutions to contribute to the selection, orientation and optimizing therapy's effectiveness monitoring, early detection of relapses for diminishing the need for further aggressive therapy, the desired effect being survival increase, preventing complications and quality of life improvement.

OBJECTIVES

The main specific objectives of the project include:

1. Expanding the knowledge related to clinico-imaging, histopathological and immunohistochemical factors involved in urothelial carcinogenesis, in order to deepen understanding and all its mechanisms;

2. Identification and definition of morphological parameters that characterize the precocious changes that occur in urothelial carcinomas, with the purpose of applying effective and differentiated therapy adapted to each case;

3. Identifying the action mechanisms of markers involved in aggression of urothelial carcinomas;

4. Studied markers involvement assessment in increase of progression and recurrence risk in urothelial neoplasia;

Achieving these objectives is based on using the following methods:

- analysis of relevant epidemiological and clinical data, analysis of imaging data, histopathological analysis for assessment of macroscopic and microscopic parameters with prognostic value in urothelial bladder carcinomas;
- immunohistochemical analysis will consider evaluation of the involvement of cell cycle markers in urothelial carcinogenesis: EGFR, HER2/neu, Ki67, p53, Bcl-2, Bcl-6.

- statistical analysis of the obtained results for noticing statistically significant correlations between clinico-pathological parameters evaluated by analyzing primary data, descriptive statistics, calculation of confidence intervals and other basic indicators (means, standard deviation, coefficient of variation, median, quartiles, etc.)

- the comparison of obtained results with those existing in medical literature.

**MATERIAL AND METHODS**

The study material was represented by patients hospitalized in the Urology Department of the Clinical Emergency County Hospital of Craiova and was conducted over a 3-year period (1 January 2009 - 31 December 2011).

This study included a total of 225 cases diagnosed with urinary bladder urothelial carcinoma which were analyzed by clinical, imaging, histopathology and immunohistochemistry methods, and the obtained data were registered in a personal record.

**RESULTS**

- Casuistry analysis by calendar years for the mentioned time interval recorded an ascending trend, most cases were registered in the last year of the studied interval, respectively in the year of 2011, accounting for 39.55%.

- The average age of patients was 59.35 years, most cases diagnosed in the 6th and 7th decade of age (65.3%), with prevalence of neoplasia in males (85.77%), the male/female ratio being 6.03:1.

- The most important risk factors for urinary bladder carcinogenesis are smoking (46.66%), the smokers/non-smokers ratio diagnosed with urothelial carcinoma of the bladder being 2.1:1, professional exposure, chronic lithiasic cystitis and nutrition.

- Disease history analysis allowed the identification of symptoms, main symptoms being macroscopic haematuria in a number of 154 cases (68.44%), frequency - 82 cases (36.44%), dysuria - 56 cases (24.88%) and combinations thereof - 19 cases (8.44%).

- Imaging diagnostic assessment of urinary bladder transitional carcinoma was performed by ultrasonography, which showed the existence of pseudotumoral formations in 83.52% of cases, in patients with suggestive symptoms, later confirmed by cystoscopy which allowed the assessment of location, shape and tumor number in 225 patients. Endoscopic electroresection was performed after cystoscopy for biopsy or curative purposes.
- We found that tumors were most often unique, located in all areas of the bladder, the bladder trigone site was dominant (28.88%), the least frequent site being the bladder dome (9.77%). Most tumors were polypoid - 116 cases (51.55%), followed in frequency by the fungoid - 63 cases (28%) and infiltrative ones - 46 cases (20.44%).

- Histopathological study of the 225 urinary bladder transitional carcinoma sought to evaluate several parameters: type and histological grade, pattern of invasion, stromal reaction, vascular and perineural invasion, tumor staging: depth of invasion (T), lymph-nodes (N) and distant metastases (M).

  It may be noted that typical forms were the most common forms of urothelial carcinomas (63.5%), most of them having papillary architectural pattern (70.6%). Histological variants of urothelial carcinomas were the urothelial carcinomas that besides the typical issues associated squamous differentiation areas (69.5%), glandular, clear cell, micropapillary and microchistic. Neoplasia study according to the degree of tumor differentiation allowed their classification into three categories: well differentiated (27.5%), moderately differentiated (55.5%) and poorly differentiated (17%).

  Analysis based on the pattern of tumor invasion allowed their classification into one of the following categories: with nodular invasion pattern (34.6%), with trabecular invasion pattern (19.1%), with infiltrative invasion pattern (23.1%), with nested invasion pattern (2.7%) and mixed invasion pattern (20.5%).

  Stromal reaction was present alone or in combination, the most common appearance being the retraction of stroma surrounding tumoral islands (55.1%).

  Analysis of tumor invasion depth, lymph node or distant metastases could be achieved for a total of 180 cases, selected from the 225 cases included in the study, of which 71 pieces obtained through cystectomy and 154 fragments obtained by TUR. The 180 cases in terms of growth pattern corresponded to transitional carcinoma with papillar pattern in 156 cases and with nonpapillary pattern in 24 cases.

  - Immunohistochemical study was performed on a total of 52 cases of primitive papillary urothelial carcinoma of the bladder of which 17 cases were well-differentiated, 23 cases were moderately differentiated and 12 were poorly differentiated cases.

  We followed the expression of markers involved in cell cycle regulation: EGFR, HER2/neu, p53, Ki67, Bcl-2 and Bcl-6, and also the correlations with clinical and pathological factors studied.

  **EGFR** expression, positive in 57% of cases, namely all poorly differentiated tumors (PD) and almost all those in stage III of disease, was missing in all well and moderately
differentiated cases (WD and MD). **Her2/neu** was positive in 46.1% of cases, most of which being PD in advanced stages and negative for WD carcinomas in stage I of disease. **Ki67** was positive in 94.2% of cases and negative in only a few cases of WD tumors in early stages of disease. **p53** was positive in 63.4% of cases. **Bcl-2** was positive in 34.6% of cases, namely in the WD and MD, and negative in PD cases in stage III of disease. **Bcl-6** was positive in 50% of cases, most WD and MD in stage I and II of disease. Only one PD case was found positive and three positive cases in the stage of disease. **ANOVA** test showed significant differences between the mean values of Ki67 depending on EGFR score, Her2/neu, p53 and Bcl-2. **Chi-square** test showed significant differences of EGFR score depending on Her2/neu score and of Bcl-2 score depending on the Bcl-6 score.

**DISCUSSIONS**

In this chapter personal results are reported to those already existing in the specialized medical literature whose authors have addressed the same topic, highlighting and detailing the ones who have relevant aspects in the study.

The average age of lesions diagnosis was assessed at 65, for the year 2005 worldwide. More recent studies, performed on 658 tumors show similar values, the average age of patients with this diagnosis being 61.97 +/- 12.97 years (range 20-90 years).

In our study investigating cases by age showed that age of patients with urinary bladder urothelial tumors ranged between the 3rd and 9th decades of age. Most cases were diagnosed in the 6th and 7th decade within which we found 147 cases representing more than half of the studied cases, or 65.3%. The average age of patients was 59.35 years.

The incidence of cases diagnosed each year showed an upward trend, most cases being registered in 2011 (89 cases, 39.55%).

Cystoscopy was a mandatory step in the diagnosis protocol of bladder tumors, confirming the suspected diagnosis raised by ultrasound and then allowing the taking of biopsies.

Typical forms were most frequently papillary-type ones in a number of 101 cases which represented 70.6% of the studied cases, unlike nonpapillary forms that were present in 42 cases and accounted for only 29.4% of cases. There were also variants with divergent differentiation in 82 cases. Literature studies in this area of interest have reported similar aspects, most urothelial neoplasia having an exofitic growth pattern associated with a good prognosis, but with high relapse rate.
The most common differentiation observed in the study was of squamous type, which constituted more than half of urothelial carcinoma tumor variants, followed by glandular type. Similar literature data communicate that squamous differentiation is the most common version of urothelial carcinoma, recent reports communicating its presence in 16.8% - 22.1% of cases.

Immunohistochemical analysis of biomarkers (EGFR, HER2/neu, Ki67, p53, Bcl-2, Bcl-6) provided information on molecular pathogenesis of bladder urothelial carcinomas, those involved in cell cycle and cellular apoptosis/proliferation being more often a study case. These can be combined in nomograms of prognosis used in order to obtain additional predictive information.

CONCLUSIONS

The incidence of transitional cell bladder carcinoma had an upward trend, most cases were diagnosed in the 6th and 7th decades of age, predominantly in males, the male/female ratio being 6.03:1, subjects to various risk factors such as smoking, professional exposure, chronic inflammation of the bladder.

The main clinical sign to guide diagnosis was haematuria, but its absence couldn’t exclude the diagnosis of bladder cancer.

The imaging study began by ultrasound examination, which was mandatory in all patients and increased suspicion of bladder cancer diagnosis raised by clinical examination and anamnesis, to be then confirmed by cystoscopy in 83.52% of patients representing 225 cases that were included in the study.

The histopathological study conducted on the 225 urinary bladder urothelial carcinomas regarding the histological-type factor showed that most of urothelial carcinomas analyzed were typical forms in 63.5% of cases and only in 36.5% of cases were variants of typical forms associating divergent differentiations.

The immunohistochemical study was performed for a total of 52 cases of papillary urothelial carcinomas which covered all stages and grades of tumor differentiation; we followed the expression of markers such as EGFR, HER2/neu, Ki67, p53, Bcl-2 and Bcl-6 involved in cell cycle regulation.

EGFR expression analysis showed positive reaction in 55.7% of cases, corresponding to all poorly differentiated tumors regardless of tumor stage, to 65.2% of moderately differentiated tumors and to 13.3% of well differentiated tumors; expression analysis
depending on the tumor stage showed positivity for all tumors in stage III, 71.4% of tumors in stage II and 22.2% of stage I tumors.

The study of Her2/neu immunoexpression showed positivity for 26 cases, representing 46.1% of analyzed cases, most positive tumors being poorly differentiated and in an advanced stage of disease.

The study of Ki67 immunoexpression showed positivity in 49 carcinomas which represented 94.2% of casuistry, negative cases corresponding to some well differentiated superficial carcinomas (stage pT1).

The study of p53 immunoexpression showed positivity in 63.43% of cases, from which 51.8% of stage I tumors, 71.4% of stage II carcinomas and 81.8% of stage III tumors.

High Ki67 and p53 indexes increased along with the degree of differentiation of urothelial papillary carcinomas.

Ki67 and p53 can be used as adjuvants on histological routine sections for the diagnosis of urothelial biopsies, being useful markers in correctly determining the diagnosis, and assessing difficult cases.

The analysis of Bcl-2 immunostaining indicated positivity for 34.6% of the analyzed tumors, the predominant forms being the well differentiated (11 cases) or moderately differentiated (7 cases) in stage I and II of disease.

Tumor growth due to antiapoptotic effect of Bcl-2 is much slower than the one determined by proliferative factors, thus the prognosis of Bcl-2 positive tumors being a better one.

The analysis of Bcl-6 immunostaining showed positivity for 50% of the investigated tumors, predominantly in well differentiated forms and rarely in the poorly differentiated ones, suggesting that Bcl-6 protein expression plays a role in transitional carcinoma differentiation.