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Histopathological Study on Patients with Transitional Cell Carcinoma of Urinary Bladder in Al-Diwaniyah City/ Iraq

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دراسة نسجية مرضية للمرضى المصابين بسرطان الخلايا الانتقالية للمثانة البولية في مدينة الديوانية/ العراق

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ألخلاصه:

أجريت هذه الدراسة على المرضى المصابين بسرطان الخلايا الانتقالية للمثانة البولية والراقدين في مستشفى الديوانية التعليمي للفترة من كانون الثاني إلى حزيران ٢٠١٠. اظهر الفحص النسجي المرضي لالتهاب المجاري البولية سمك اعتيادي للخلايا ألطلائية للمثانة البولية والذي كان حوالي(٤-٦) خلايا، إضافة إلى وجود احتقان في الطبقة تحت المخاطية والأنسجة العضلية. كما أظهرت الدراسة الحالية إن سرطان الأنسجة الانتقالية للمثانة البولية احتوى ثلاثة تدرجات رئيسة : الدرجة I، الدرجة II ، الدرجة III. تم استخدام cytokeratin 7 كمعلمات للأورام لتأكيد تشخيص مرض سرطان المثانة البولية، إذ أظهرت النتائج وجود نتائج موجبة وسالبة بين معلمات السرطان والخلايا السرطانية.

Abstract:

This study conducted on inpatients admitted to Al-Diwaniyah teaching hospital at period from January to June 2010 to recognize the histopathological changes of tumor cells of urinary bladder in comparison with normal cells. The histopathological examination of urinary tract infections (UTIs) showed normal thickness of urothelium, in addition, the submucosa and the muscular tissue were congested and oedematous. The present study showed that the transitional cell carcinoma of the bladder divided into three major grades: Grade I , Grade II, Grade III. In this study, the immunehistochemical expression of cytokeratin 7 used to confirm the diagnosis of urinary bladder cancer. The results revealed a positive and negative reaction between tumor cells and cytokeratin7 antibody.

Introduction:

The bladder is a hollow, muscular, balloon-like organ that stores urine. It sits in the lower part of the abdomen. Urine consists of water and waste products not needed by the body[1] . The bladder is lined with epithelial tissue that stops the urine going into the body. The cells of this tissue are called transitional cells or urothelial cells. The lining epithelial tissue is called the urothelium. The kidneys produce urine, which is carried to the bladder by tubes called ureters[2].

Nearly all cancers of the bladder begin in the urothelium[3]. These are called transitional cell cancers (or urothelial cancers). They come in a wide range of forms and can behave in very different ways. Transitional cell cancers grow from the inside lining of the bladder. They are either superficial or invasive cancers[1]. Most bladder cancers are superficial cancers. They either look flat and red (carcinoma in situ) or stick out from the lining like mushrooms (papillary). Superficial cancers do not often spread to other parts of the body [4].

Less often, transitional cell cancers grow deeply into the wall of the bladder. These are called invasive cancers and are more likely to spread to other parts of the body

The common forms of bladder cancer include transitional cell carcinoma, squamous cell carcinoma, and adenocarcinoma.

Bladder tumor markers are chemical or immunologic tests to detect specific substances bladder cancer cells release into urine [5]. Although some newer tests are available to more closely examine the bladder cancer cells, these are not used to make treatment decisions outside of a clinical trial.

Cytokeratins are proteins of keratin-containing intermediate filaments found in the intracytoplasmic cytoskeleton of epithelial tissue. The term "cytokeratin" began to be used in the late 1970s when the protein subunits of keratin intermediate filaments inside cells were first being identified and characterized [5].

The bladder cancer's stage indicates how far it has spread within the bladder, to nearby tissues, and to other organs. The stage of a cancer is one of the most important factors in selecting treatment options, as well as the most significant (but not the only) factor in predicting prognosis[6] .

The aim of study is to examine the histopathological changes of tumor cells of urinary bladder in comparison with normal cells and use Immunehistochemistry technique(using cytokeratin 7) as tumor marker to confirm the diagnosis of urinary bladder cancer.

Materials and Methods

-Samples processing: Ten tumor samples(biopsies) were taken from inpatients admitted to Al-Diwaniyah teaching hospital at period from January to June 2010 who clinically diagnosed by urosurgeon as suspected patients with transitional cell carcinoma. The biopsies were fixed in 10% neutral buffered formalin for 24 hours, The preparations was performed according to[7] . After fixation, the tissues were trimmed and washed by tap water for 4-6 hours to remove the formalin solution

and transferred to the following steps: dehydration, clearing, infiltration with paraffin wax, and blocking. Blocks were cut using rotary microtome (Micron, Germany) in thickness(5-6) μm, two slides were prepared, the first one was prepared for Haematoxylin and Eosin procedure[7], while the other put on marker slide which was prepared for immunehistochemistry and staining of tumor tissues which were performed to confirm the diagnosis of bladder cancer. Cytokeratin7 antibody (CK7, 1/250 dilution, DAKO-Corp) was used according to [8] which was expressed as brown stain inside the cytoplasm.

Results and Discussion:

Histopathological study of urinary bladder :

Urinary tract infection (non tumor urothelium)

The histopathological examination of UTI showed normal thickness of urothelium which was about (4-6) cells (Fig.1). In addition, the submucosa and the muscular tissue were congested and oedematous (Fig.2). On the other hand, a number of inflammatory cells were infiltrated including lymphocytes and other cells. This findings are agreed with [9] and [10] who pointed that the histological features of UTI reflect the inflammatory process and are usually non specific.

Histopathological changes on urinary bladder suffering bladder carcinoma:

The present study of transitional cell carcinoma of the bladder were divided into three major grades as following:-

Grade I: in this grade, the histological examination showed the presence of papillary projections which were composed of fine branching fronds each of which has a thin central core of a vascular connective tissue and lining the epithelium which was 4-6 cells thick(Fig.3) and resembles very closely the normal transitional epithelium of the urinary bladder (Fig.4). In addition , the results revealed that the transitional epithelial cells showed minimal nuclear pleomorphism and mitotic activity. The papillae were long and delicate and the fusion of papillae was limited.

The current result concerning grade I was in parallel with [11] and [12] who mentioned that the histological and cytological features are of the best differentiation in the Papilloma of bladder.

Grade II: in this intermediate grade, there were more layers of cells which increased in growth (Fig.5). The histopathological examination revealed that the tumor cells tended to be crowded more tightly together and showed an array of different cytological features (Fig.6). It was greater variety in size, shape and polarity(pleomorphism)and denser, coarser chromatin in staining (nuclear hyperchromatin). The results also showed the nucleoli of tumor cells were abnormally large, numerous, and intensely staining. The mitotic activity was higher than grade I. On the other hand, there was no invasion to the blood vessels (Fig.7) and muscular tissue (Fig.8).

The microscopic examination of this grade was similar to that described by [13] and [14], but it was different to that mentioned by [15] who suggested that the invasion of the underlying bladder wall may occur with any grade of transitional cell carcinoma.

Grade III: the histopathological examination of transitional cell carcinoma of the bladder in this grade showed the papillary areas were rare and the fusion and blunting were typical (Fig.9). Also, there were significant and marked nuclear pleomorphism (Fig.10). The nucleoli of tumor cells were larger, intensely stained and more numerous than that of other grades.

The present results showed important characteristics features concerned with the invasion of the underlying tissue of bladder wall which included the muscular tissue (Fig.11) and blood vessels (Fig.12).This result agrees with [16] who found that the epithelial lesion composed of cells which are poorly differentiated and showed highly mitotic activity. On the other hand, [17] suggested that the invasion capacity is confined to this grade which is consistent with current study.

3.1.6. Immunehistochemical Expression Cytokeratin7 tumor marker in tumor cells :

This test was performed to confirm the diagnosis of bladder cancer using tumor marker (cytokeratin7). The cytokeratin 7 was expressed in malignant cells of urinary bladder as brown stain as showed in figure(13). The positive reaction is illustrated in Figure (13) which was grade III of transitional carcinoma of bladder. Positive Immunehistochemical reactions were as either fine granules or diffuse dark brown-red coloration in the cytoplasm, our finding is correlated with finding of [18]. This staining was observed more or less in all layers of transitional epithelium . whereas often in the outer layer of epithelial cells (Fig.13). In general, cytokeratin displayed the most prominent and diffuse immunostaining.

The negative reaction between tumor cells and tumor marker is illustrated in figure (14)(no brown coloration is observed).

Spontaneous bladder tumors are often occurring in humans[19]. The biological outcomes of such tumors are estimated by novel histopathological methods. As used for other tumors, Immunehistochemical methods can also be used for bladder tumors diagnosis and to determine the activation of cell proliferation and their invasiveness or to evaluate the cell cycle related proteins and define the type of tumors [20]. One of the tumor markers used in this study is cytokeratin, which is an intermediate filament present in epithelial cells. The cells contain at least 20 different cytokeratins[21]. cytokeratin is helpful to differentiate the cell origin of carcinomas including urothelial tumors [22]. In fact, the positivity defined in cytokeratin is 70%-95%. However, cytokeratin staining can also presented in benign epithelium, but this staining is limited to surface “umbrella” cells where the staining expressed in the whole cell of dysplastic or malignant epithelium.

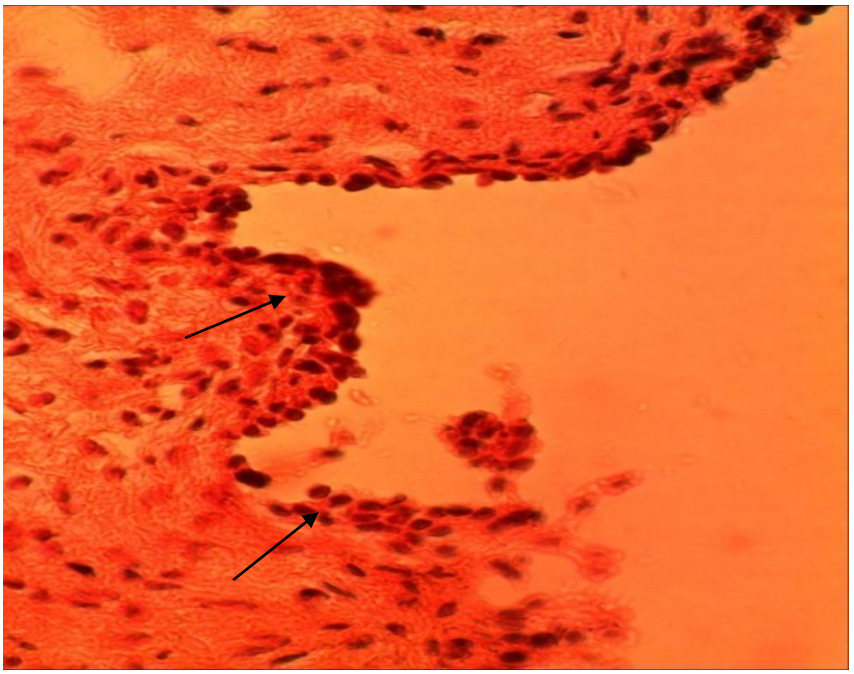
However , In this study, a more intense and severe diffuse reaction was obtained with cytokeratin in transitional cell carcinoma.

Conclusions:

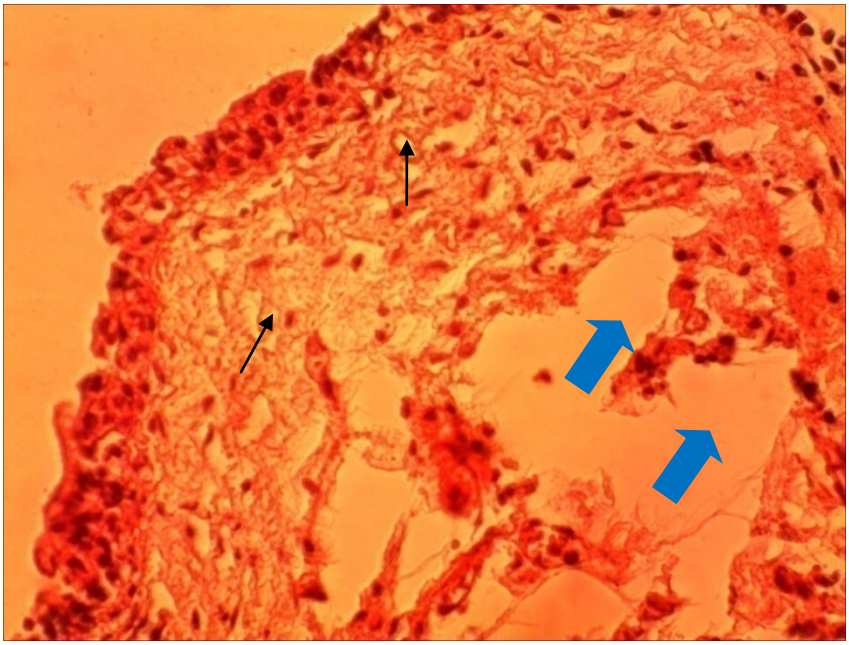
1. The observation of some histopathological changes in the examined tissues of urinary bladder especially in cases of bladder cancer in comparison with urinary tract infection alone.
2. The use of immunohistochemistry test gave a good and precise marker to diagnose the tissues cancer in comparison with Eosin and Heamatoxylin technique.

Recommendations :

1. Early diagnosis and treatment of UTIs in order to avoid the development of bladder cancer as a result of chronic irritation.
2. Application of another type of tumor marker as CK20 and CEA in order to diagnose other types of tumor as colon and lung cancer.



Figure(1): Histopathological section of bladder shows normal thickness of urothelium(⟶),(H&E stain X 400)



Figure(2): Histopathological section of bladder shows Congested blood vessels(↑), and Oedema in submucosa (⬆).(H&E stain X 400)

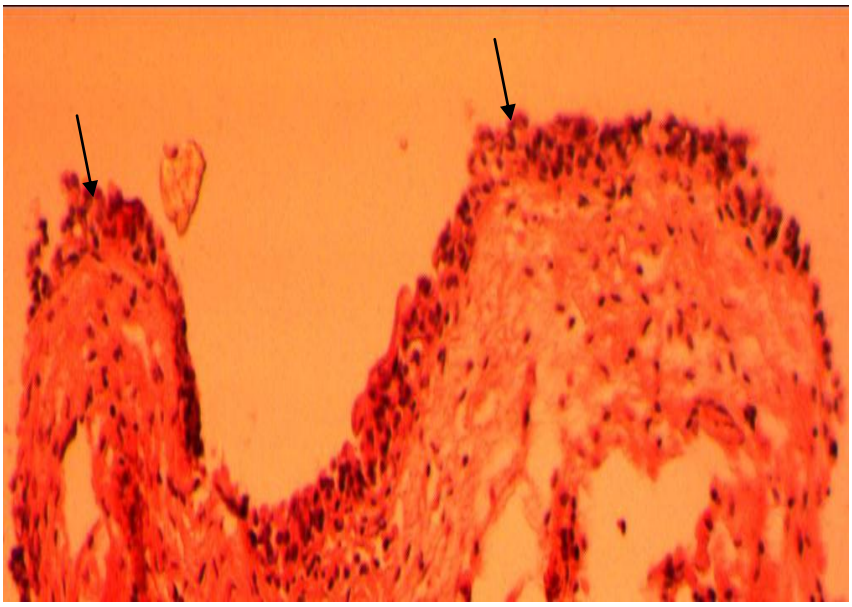
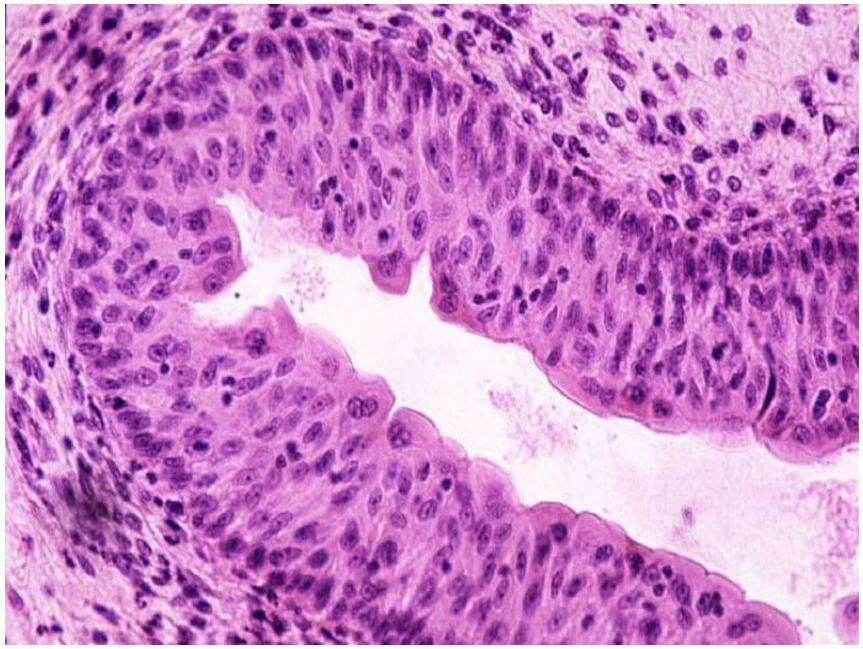
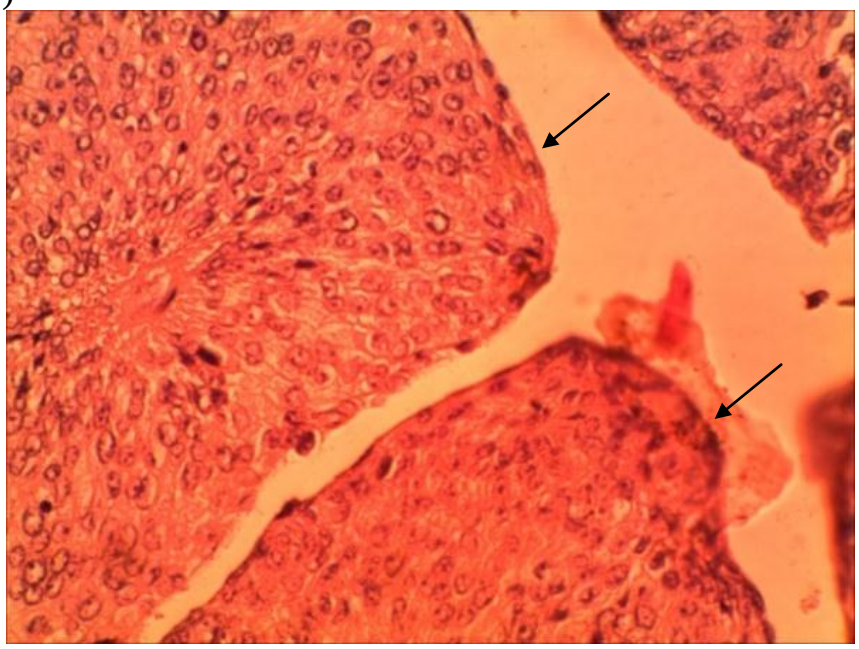


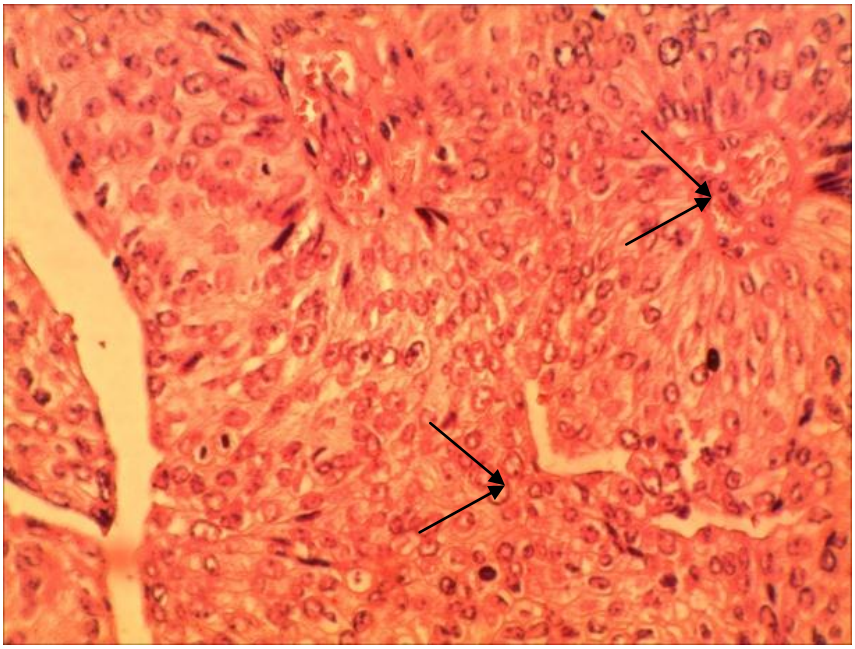
Figure (3): Histopathological section of urinary bladder shows papillary projections(⟵)of grade I of transitional cell carcinoma (Grade **I)**. (H&E stain X 100)



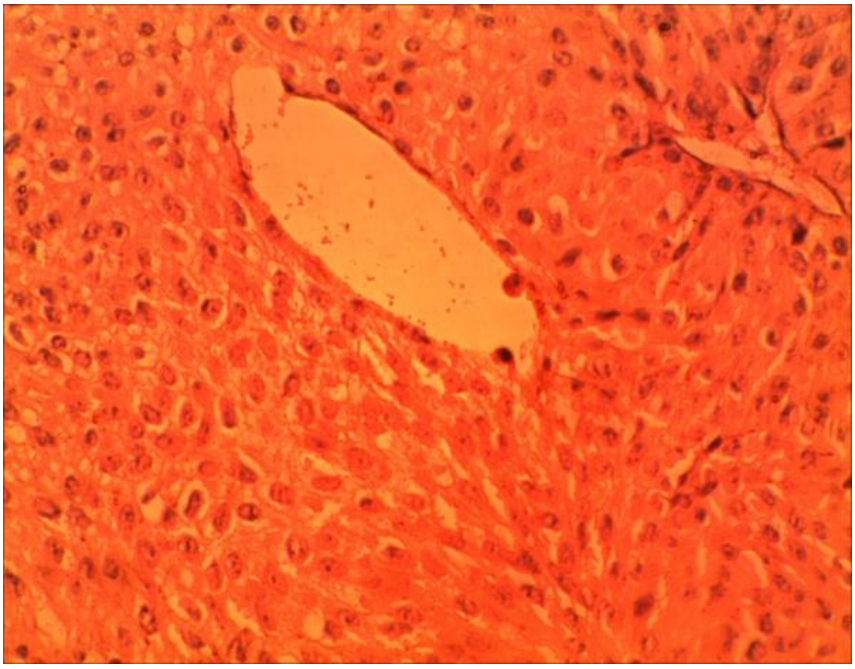
Figure(4): Histological section of urinary bladder shows the normal transitional epithelium of the urinary bladder.(H&E stain X 400)



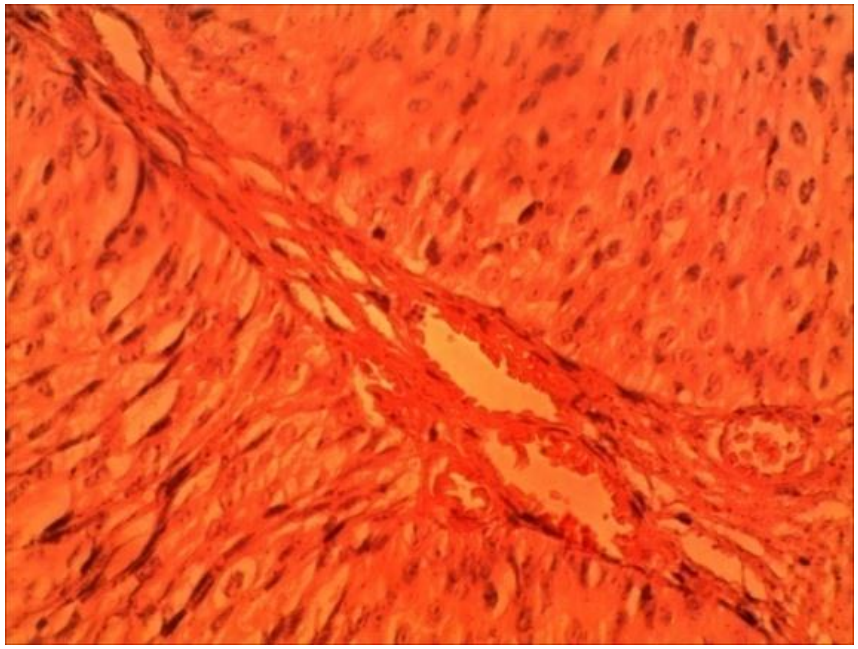
Figure(5): Histopathological section of urinary bladder shows more layers of cells covering the papillae(⟵) which increase in growth(Grade **II)**.(H&E stain X 400)



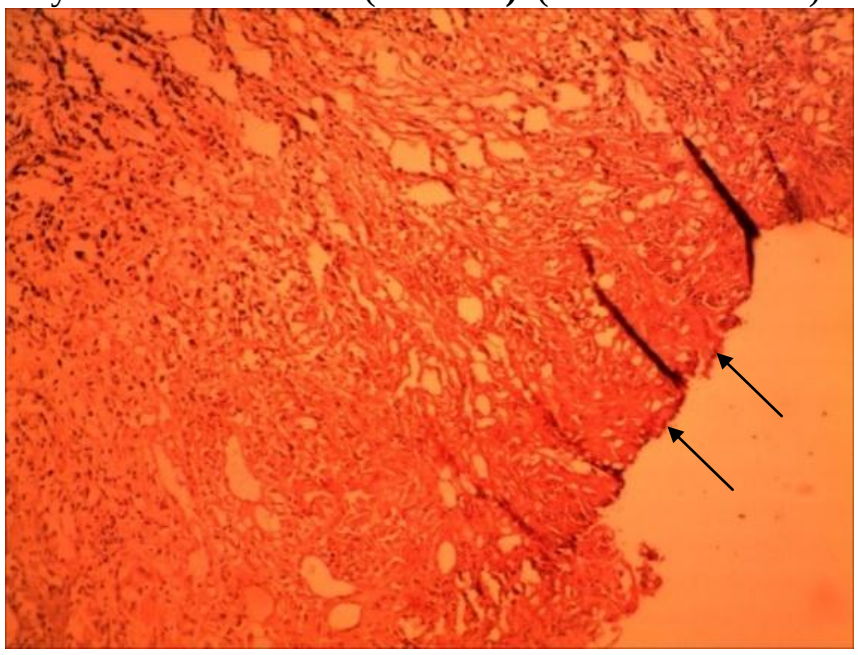
Figure(6): Histopathological section of urinary bladder shows crowded tumor cells(⟶) and an array of different cytological features(Grade **II)**. (H&E stain X 400)



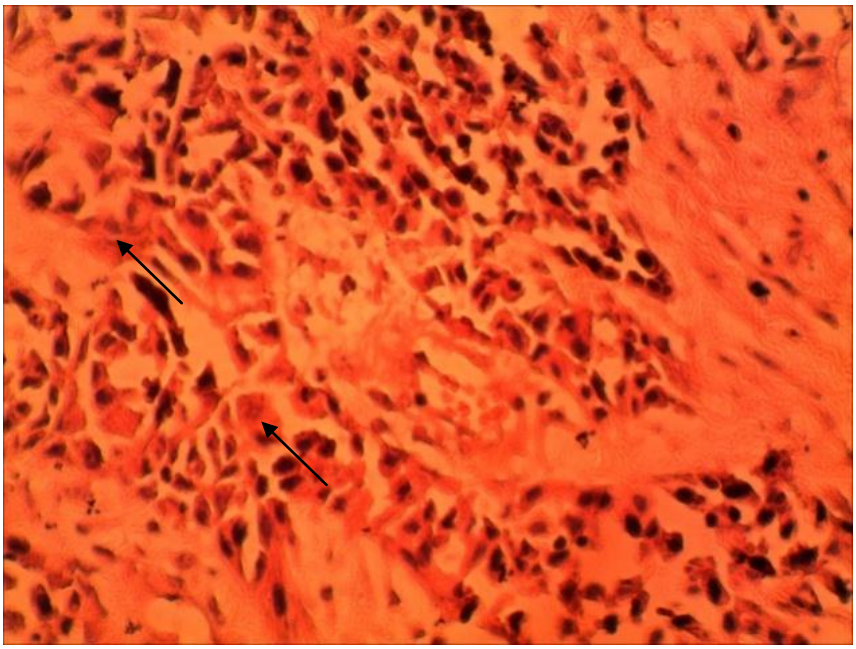
Figure(7): Histopathological section of urinary bladder shows no invasive capacity to blood vessels(Grade **II)**. (H&E stain X 400)



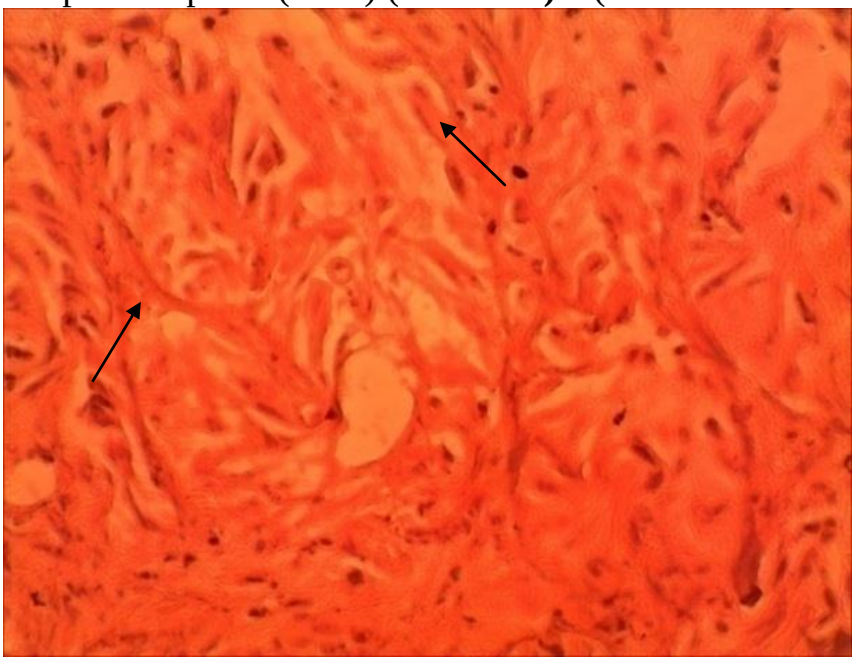
Figure(8): Histopathological section of urinary bladder shows no invasive capacity to muscular tissues(Grade **II)**. (H&E stain X 400)



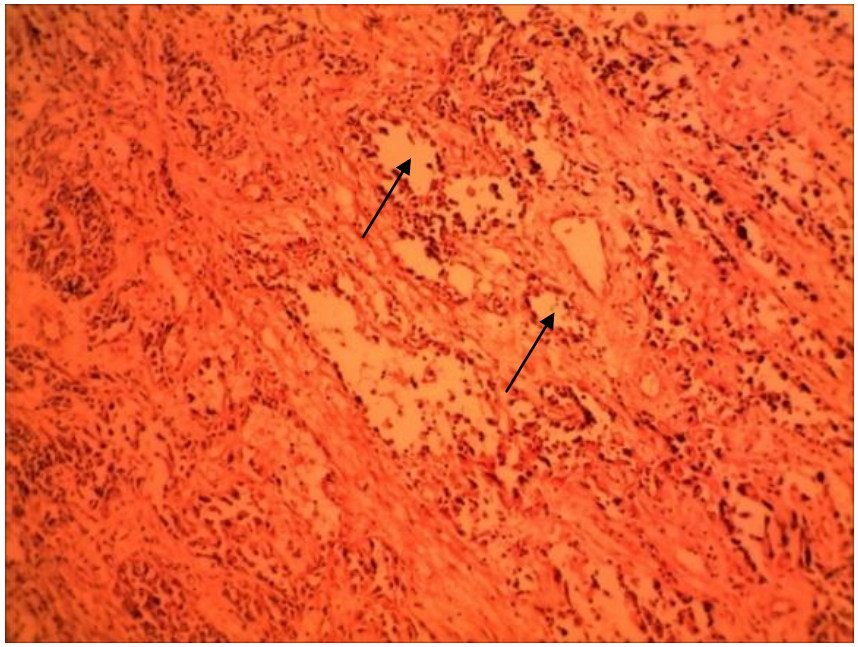
Figure(9): Histopathological section of urinary bladder shows fusion and blunting of papillae(⟵) (Grade **III)**. (H&E stain X 100)



Figure(10):Histopathological section of urinary bladder shows marked nuclear pleomorphism(⟵) (Grade **III)** . (H&E stain X 400)



Figure(11):Histopathological section of urinary bladder shows invasive capacity of muscular tissue(⟵) (Grade **III)**. (H&E stain X 400)



Figure(12):Histopathological section of urinary bladder shows invasive capacity of blood vessels(⟵) (Grade **III)**.(H&E stain X 100)

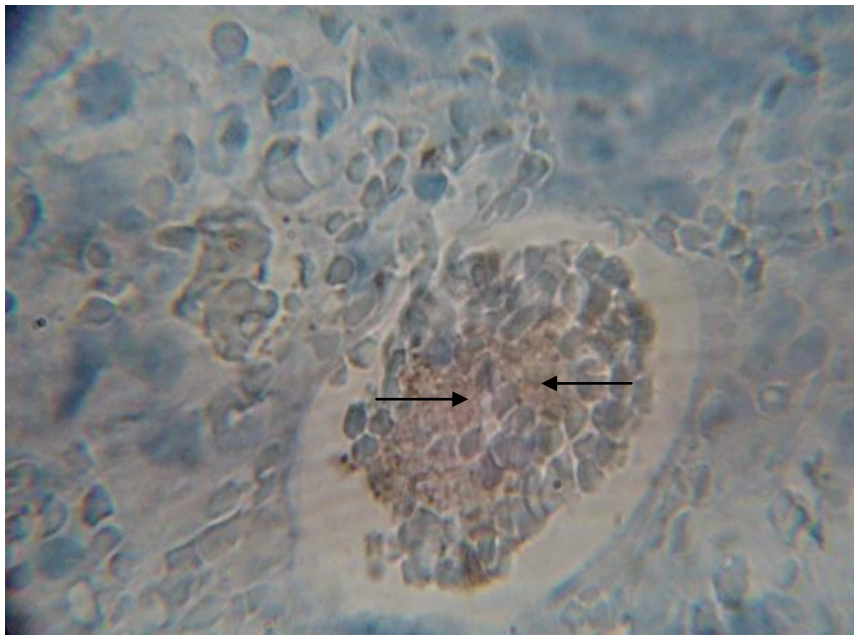


Figure (13) : Immunehistochemical staining of cytokertin7 in transitional cell carcinoma shows positive reaction in malignant cells (⟵). IHC. (X 400)

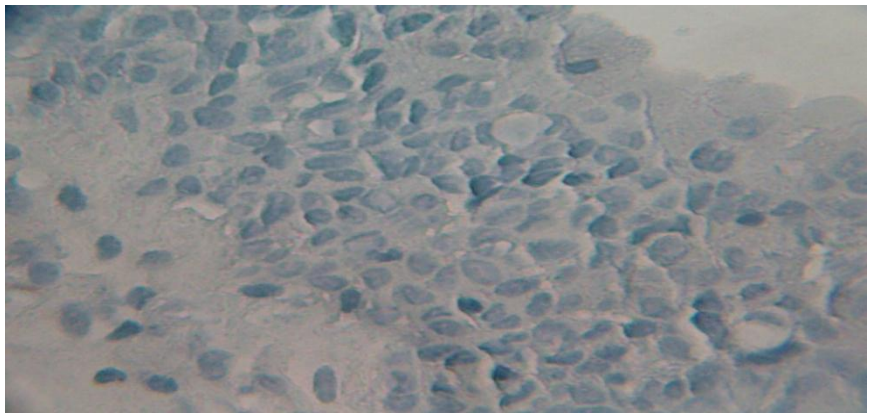


Figure (14) : Immunehistochemical staining of cytokertin7 in transitional cell carcinoma shows negative reaction in malignant cells of urinary bladder with cytokeratin 7. IHC. (X 400)

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