Scientific Research and Essays Vol. 6(26), pp. 5548-5554, 9 November, 2011
DOI: 10.5897/SRE11.1126
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 ©2011 Academic Journals

Full Length Research Paper

Role of fluorescence *in situ* hybridization in the diagnosis of urothelial cancer in asymptomatic hematuria patients

Peng Wang, Feng-shuo Jin*, jin Ye, Ke-qin Zhang, Gang Wu, Yao Zhang and Yan-feng Li

Department of Urology, Research Institute of Field Surgery, Daping Hospital, Third Military Medical University, Chongqing 400042, China.

Accepted 24 August, 2011

The present study aimed to investigate the feasibility and validity of fluorescence *in situ* hybridization (FISH) in the diagnosis of urothelial cancer (UC) in asymptomatic hematuria patients. Probes to the pericentromeric regions of chromosomes 3, 7 and 17, and to the 9p21 band were used in FISH at detecting UC in 53 hematuria patients negative on imaging, cystoscopy and cytology but highly suspicious for UC. Among 53 asymptomatic patients with hematuria, 15 were FISH-positive, of whom 13 were followed for 3-12 months. Of these 15 patients, 5 were finally diagnosed with bladder UC and 2 with renal pelvis cancer. Among 38 patients who were FISH-negative, none developed UC during the follow up period. FISH is a significant additional and complementary method for detection of UC in hematuria patients who were negative on cystoscopy and cytology but suspicious for UC.

Key words: Bladder cancer, *in situ* hybridization, diagnosis.

INTRODUCTION

Hematuria is a common clinical symptom and patients with hematuria should receive comprehensive examination regardless of age, gender and clinical symptoms which may be helpful to identify the potential malignant tumor, especially in asymptomatic hematuria patients with early stage urinary tract cancers. The urinary cancers at early stage and of low grade have good prognosis if timely treated. In a study, researchers examined 200 asymptomatic hematuria patients aged 40-50 years regularly and results revealed that 11% of them were finally diagnosed with bladder cancer (Liu et al., 1999). Early diagnosis of urothelial carcinoma (UC) is a key to improve therapeutic efficacy and decrease mortality. However, to date, no effective strategy has been developed for early diagnosis of UC. Cystoscopy or ureteroscopy followed by pathology have been considered as the gold standard in the early diagnosis of UC. Nevertheless, for patients with microscopic hematuria and those with chronic bladder irritation, these methods have low positive rate and low positive predictive value. Moreover, these methods are invasive and patients usually have poor compliance to these examinations, which results in misdiagnosis or delayed diagnosis. In addition, imaging examinations are also difficult to identify the lesion smaller than 0.5 cm. In recent years, with the understanding of the genetic alterations of UC, studies have demonstrated that partial or complete deletion of the P16 gene at 9p21 is one of the most common alterations in UC, and the occurrence and development of UC are closely related to the aneuploidy of chromosomes 3, 7 and 17 (Sokolova et al., 2000; Halling et al., 2000). In the present study, fluorescence *in situ* hybridization (FISH) was employed to detect the UC in hematuria patients suspicious for UC, which aimed to explore the value of FISH in the diagnosis

*corresponding author. E-mail: jinfs5505@163.com.

Abbreviations: FISH, fluorescence in situ hybridization; **UC,** urothelial cancer; **KUB+IVU,** kidney-ureter-bladder and intravenous urography; **BCG,** Bacillus Calmette-Guerin; **BTA,** bladder tumor antigen.

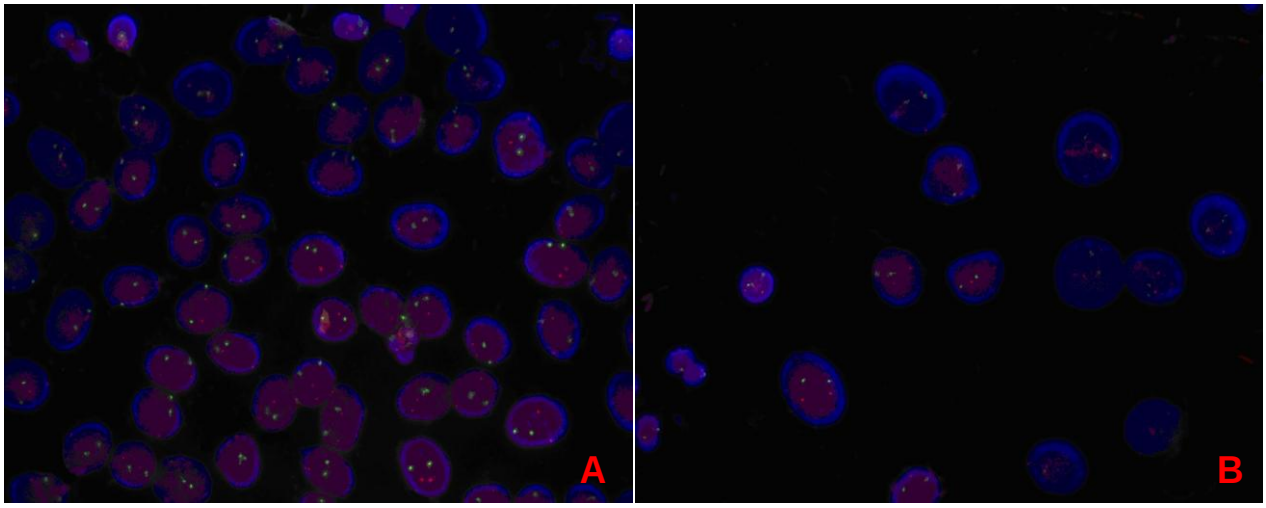

Figure 1. Fluorescence *in situ* hybridization of normal cells (DAPI; ×100). **A**, After hybridization by probes to the pericentromeric regions of chromosomes 3 and 7 (DAPI×100); **B**, after hybridization by probe to the pericentromeric region of chromosome 17 and probe to P16 region (DAPI×100).

of UC in asymptomatic hematuria patients.

PATIENTS AND METHODS

Characteristics of patients

A total of 53 hematuria patients who were negative on imaging of urinary tract, cytology and cystoscopy and suspicious for UC were recruited into the present study. There were 39 male and 14 female with the age ranging from 33-85 years (mean age: 51±8 years). Our study has been approved by the Ethics Committee of Daping Hospital affiliated to Third Military Medical University.

Sample collection

Samples were collected from patients from February 2008 to February 2010.

Criteria for diagnosis

All patients underwent urinary tract ultrasonography, cytology and FISH. Before cystoscopy or ureteroscopy, fresh voided urine was collected for consecutive 3 days (300 ml each) and divided into two: one was used for cytology and FISH. The tissues obtained from cystoscopy and ureteroscopy were processed for pathological examination followed by analysis by 3 pathologists independently and the consensus was then achieved.

Methods

Probes to the pericentromeric regions of chromosomes 3, 7 and 17, and to the 9p21 band were purchased from Beijing GP Medical Tech Co., Ltd. The probes to chromosomes 3 and 17 had green fluorescence, and those to chromosome 7 and 9p21 (P16 probe) had orange fluorescence. The collected urines were divided into two: one was used for FISH according to manufacturer's instructions and the other for HE staining and detecting cancer cells (Wang et al., 2009).

Enumeration of fluorescence *in situ* hybridization signals and analysis of results

Under a fluorescence microscope, the images were analyzed with a FISH point counting analysis software 2.0 and a total of 100-200 cells were analyzed. The criteria for interpretation includes, monoploid proportion of nucleus with one signal>15% of total cells; triploid and polyploidy: proportion of nucleus with 3 or more signals>10% of total cells. The criteria for combined probe analysis were as follows (Halling et al., 2000): 4 or more cells showing gain of 2 or more chromosomes (3, 7, and 17) in the same cell, or loss of homozygous 9p21 locus in 12 or more cells. Once cancer cells were identified in cytology, the sample was defined as positive.

RESULTS

Results from fluorescence *in situ* hybridization

Among 53 patients with asymptomatic hematuria, 15 were FISH-positive of who 13 were followed up for 3-12 months, 5 had confirmed UC and 2 renal pelvis cancer. Of the 7 patients with confirmed cancer, there were 5 male and 2 female and all were older than 40 years. The remaining 38 patients negative for FISH did not develop cancers during the follow up period. In FISH, the normal cells had 2 green and 2 red fluorescence signals (Figure 1). The probe to the pericentromeric region of chromosome 3 and the P16 region were orange, and probes to the pericentromeric regions of chromosomes 7 and 17 had green signals. The abnormal cells had aberrant fluorescence signals: multiple signals, single signal and absence of signal which represent the number

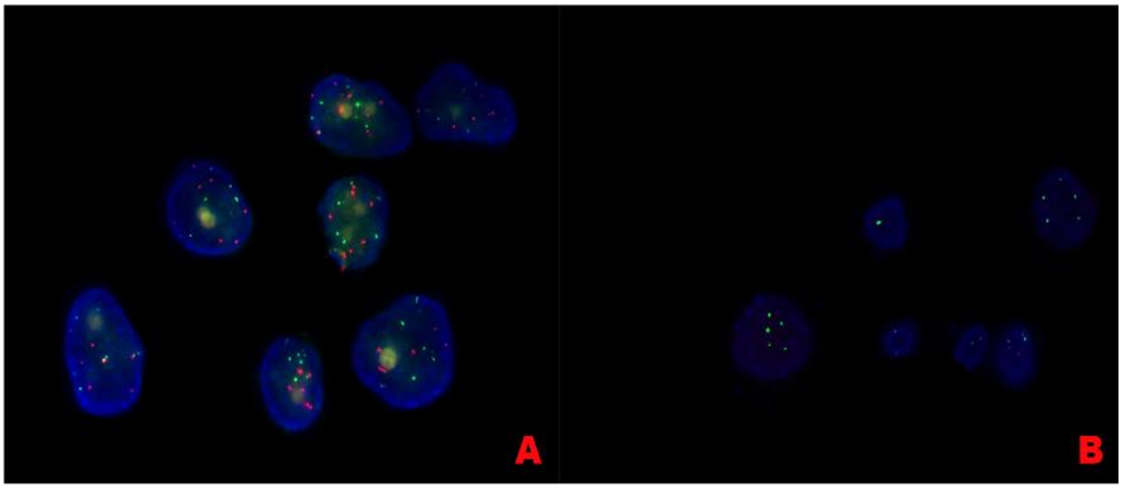

Figure 2. FISH of UC (DAPI; ×100). **A**, After hybridization by probes to the pericentromeric regions of chromosomes 3 and 7 (DAPI×100); **B**, after hybridization by probe to the pericentromeric region of chromosome 17 and probe to P16 region (DAPI×100).

of chromosome: increase, decrease and absence, respectively (Figure 2).

Descriptions of specific cases

Case 1

A 81-year old male patient with intermittent and painless macroscopic hematuria for 1 month received ultrasonography of urinary tract, plain film of kidney-ureter-bladder and intravenous urography (KUB+IVU) and cytological examination, all these examinations showed negative findings. Cystoscopy revealed scattered bleeding points in the bladder trigone and pathology indicated chronic endocystitis (Figure 3A). In the FISH, the cells display the gain of extra copies of chromosomes 3, 7 and 17 (Figure 3C). Homozygous deletion of 9p21 was also noted (Figure 3D). Bladder tissues were randomly obtained from the bladder trigone, bladder wall and bladder neck in a second cystoscopy, and the suspected lesion was also collected. Pathology showed UC in the bladder neck (Figure 3B).

Case 2

A 46-year old patient received ultrasonography of urinary system due to recurrent pain in the left waist accompanied by intermittent macroscopic hematuria for 1 year, and results showed left kidney stone and left hydronephrosis. KUB+IVU showed left kidney stone and filling defect in the lower renal calices. This patient was suspicious for space occupying lesion in the left kidney (Figure 4). CT of bilateral kidneys revealed left kidney stone and hydronephrosis, space occupying lesion in the renal pelvis which implies renal pelvis cancer (Figure 6A). However, two FISHs showed negative results (Figures 5A and B). Then, left flexible ureteroscopy was performed and showed a white floccule in the left renal pelvis measuring 0.5-0.6 cm in diameter and inflammation or tumor could not excluded. Subsequently, biopsy and pathological examination were done and results revealed chronic mucositis of left renal pelvis (Figure 7). CT of bilateral kidneys indicated left kidney stone but space occupying lesion was absent in the renal pelvis (Figure 6B).

DISCUSSION

In urinary tumor, patients with hematuria as first symptoms, has bladder cancer which is the most common type, followed by renal pelvis tumor and ureteral tumors. Similar to other malignant tumors, UC also has alterations of complicate cellular genetics and molecular genetics. The alterations of cellular genetics are mainly characterized by chromosomal aberrations including alterations of chromosome number and structure, and those of molecular genetics by activation of oncogenes as well as inactivation/deletion of tumor suppressor genes. FISH is the most frequently used tool to investigate the molecular genetics and mainly applied in the detection of chromosome number and structure. In our previous studies, FISH has relatively high sensitivity and specificity in the detection of bladder UC. In the present study, 15 hematuria patients negative on imaging examination, cytoscopy and cytology were FISH-positive. Among these patients, 13 were followed up for 3-12 months and finally 5 were diagnosed with bladder UC

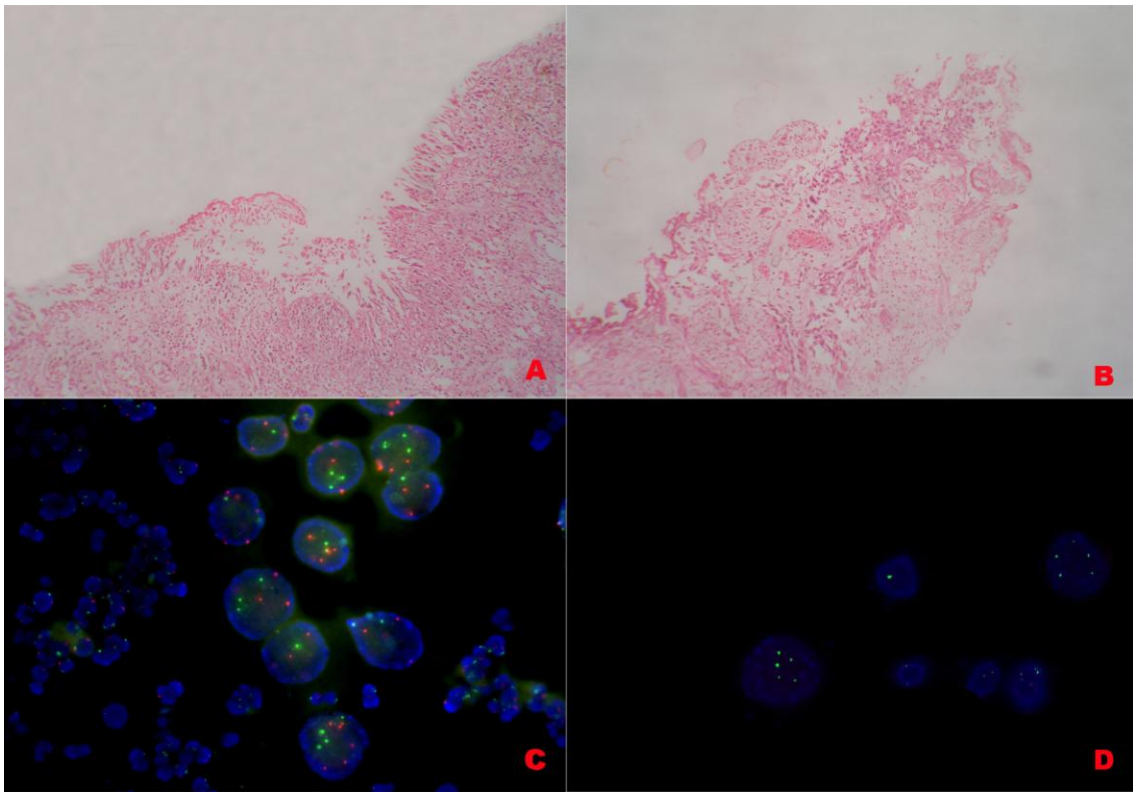

Figure 3A. First cystoscopy showed chronic endocystitis (HE×20); B, second cystoscopy showed local advanced UC (HE×20); **C**, cells displayed gain of extra copies of chromosomes 3 and 7 in fluorescence *in situ* hybridization (DAPI×100); **D**, cells displayed gain of extra copies of chromosome 17 and P16 region in fluorescence *in situ* hybridization (DAPI×100).

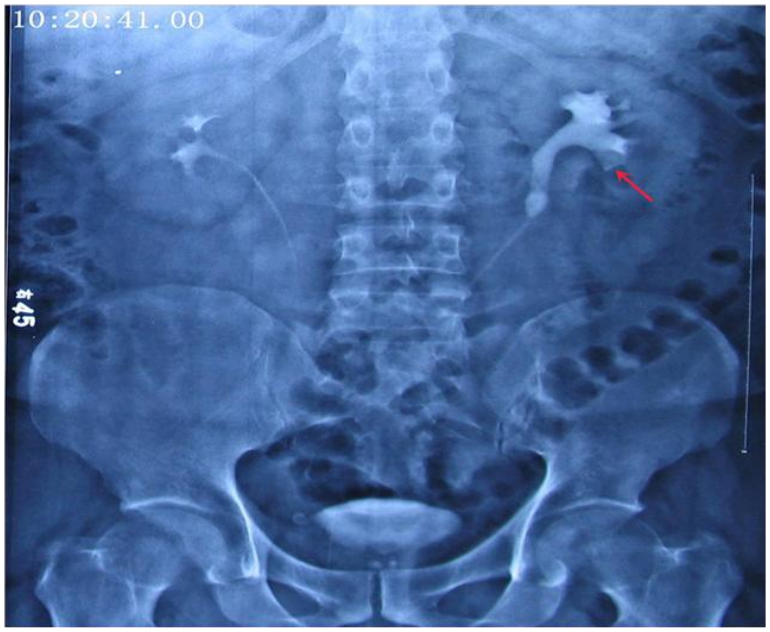

Figure 4. Kidney-ureter-bladder and intravenous urography revealed left kidney stone and filling defect in the lower renal calices, and space occupying lesion was considered (arrow).

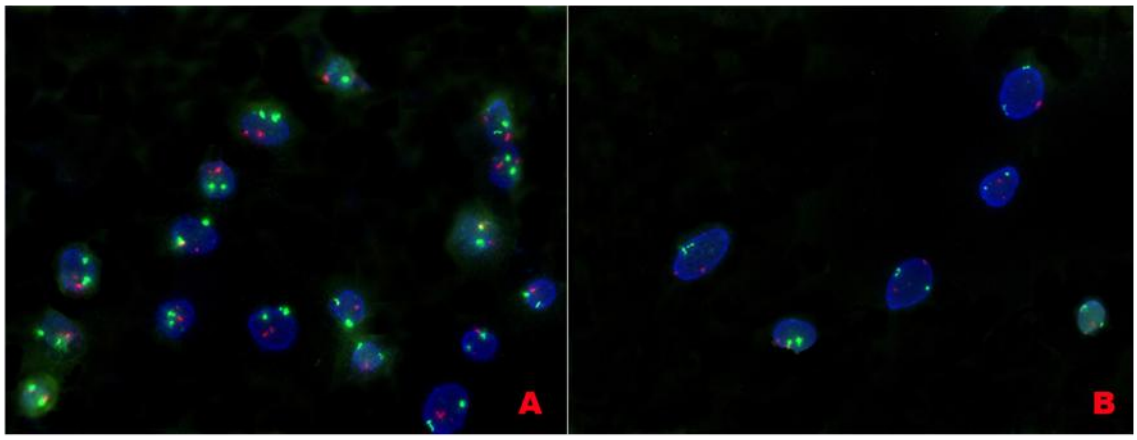

Figure 5A. cells displayed gain of extra copies of chromosomes 3 and 7 in fluorescence in situ hybridization (DAPI×100); **B,** cells displayed gain of extra copies of chromosome 17 and P16 region in fluorescence in situ hybridization (DAPI×100).

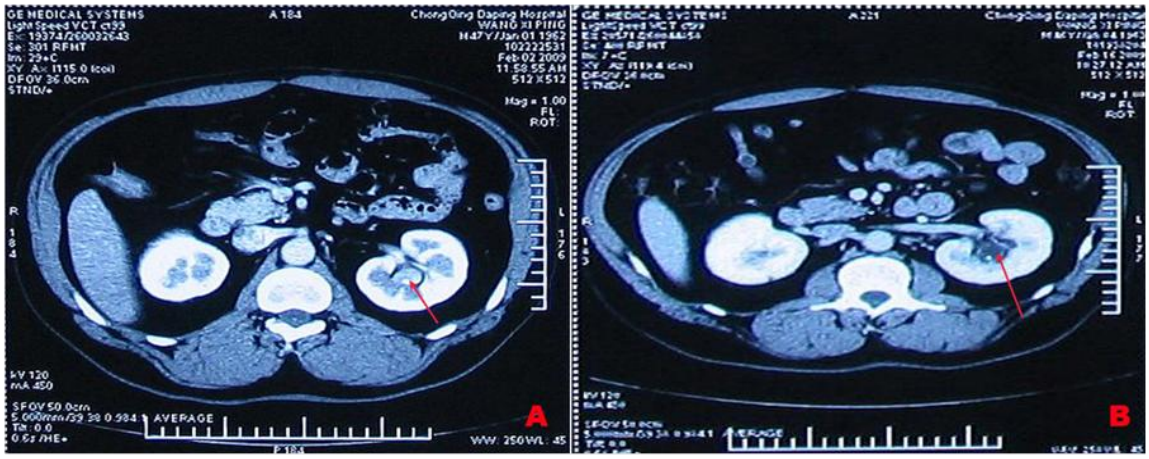

Figure 6A. CT of bilateral kidneys showed left kidney stone, space occupying lesion in the left renal pelvis suggesting renal pelvis cancer (arrow); **B**, CT of bilateral kidneys showed left kidney stone but space occupying lesion was absent in the renal pelvis (arrow).

and 2 with renal pelvis cancer. The random biopsy of suspected lesion followed by pathological examination only possesses the positive rate of 37% even this examination is done by an experienced urological surgeon. In addition, the overall positive rate of cystoscopy is lower than 10% in microscopic hematuria patients, and bladder neck region is a blind region and can not be observed in cystoscopy. Therefore, cystoscopy can not be applied as a gold standard in all patients who required biopsy and subsequent pathological examination (Sarosdy et al., 2002). Cytological examination of voided urine is a non-invasive method and has relatively high specificity but its sensitivity is still low. Our previous study (Wang et al., 2009) showed that the positive rate of cytology was as low as 20-32.5% in the detection of UC, especially in patients with tumor of low grade/early stage. In addition, cytological examination is susceptible to influence by subjective factors. For early stage bladder cancer, the cell differentiation is good and the features of these cells are similar to normal cells. Thus, these cells are difficult to identify from normal cells (Roy et al., 1992). Additionally, urine routine test and counting of formed elements of urine sediment have extremely low specificity in the detection of bladder cancer. For early bladder cancer, the bladder mucosa is nearly normal in the lesion site which can not be timely identified by cytoscopy and imaging examination. However, FISH can be used for early diagnosis of bladder cancer.

In the present study, the diagnosis of case 2 suggested

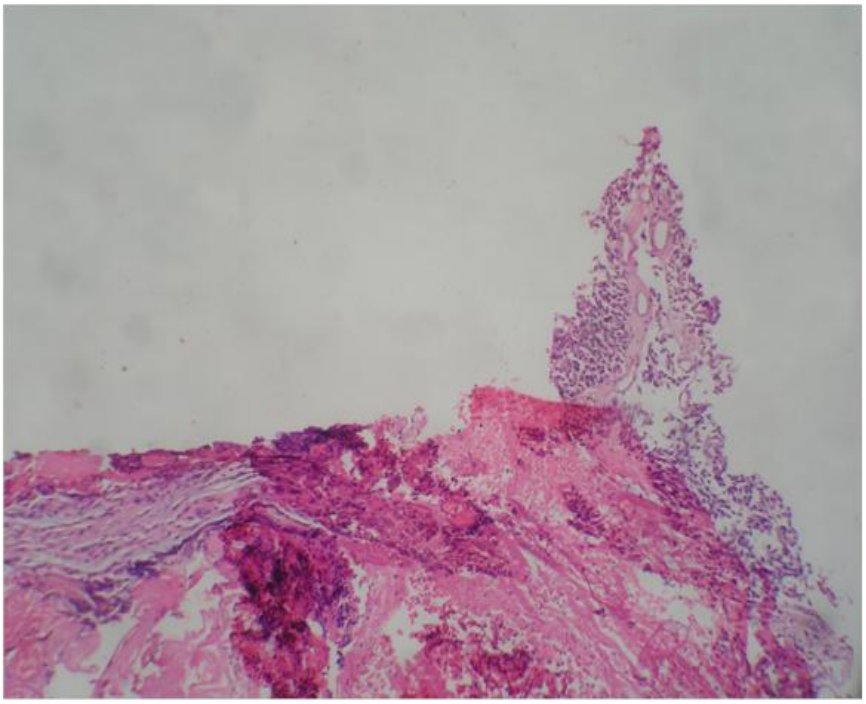

Figure 7. Pathology showed chronic mucositis in the renal pelvis (HE×20).

that FISH could be used in not only early diagnosis of urinary tumors but the early differentiation diagnosis of tumors in patients with false-positive results on imaging. Plain film of KUB+IVU cannot identify the urinary tumors from urinary inflammatory lesions. In addition, the anatomy of normal renal pelvis varies in subjects, which places difficulty in the identification by imaging examination. Abdominal ultrasonography of urinary system is not effective in the identification of tumors smaller than 0.5 cm in diameter (Wang et al., 2008). For renal pelvis tumor, the resolution in ultrasonography is limited and the echo of renal sinus is disordered. When the space occupying lesion is relatively small, the ultrasonography can not distinguish this lesion from the normal tissues. FISH can effectively identify the lesions whose clinical manifestations do not match the findings on imaging, and also provide evidence for the further clinical treatment. Therefore, the misdiagnosis and subsequent inappropriate surgical intervention are avoided.

FISH can be used to identify urothelial tumors at early stage but can not localize the tumors especially in patients negative on imaging examination and endoscopy who should be closely followed up for early localization diagnosis. However, FISH provides important clinical information. For patients positive for FISH and with suspected bladder tumor, multiple punch biopsies under cystoscopy or fluorescence cystoscopy can be done for confirmed diagnosis. In patients positive for FISH and with suspected ureteral tumors or renal pelvis tumors, the findings on imaging should be re-evaluated, and ureteroscopy or flexible ureteroscopy can be performed for the suspected lesions. In this report, in a patient positive for FISH and with long-term microscopic hematuria, we re-evaluated the features on intravenous pyelography. In the first evaluation, we proposed that the small filling defect was attributed to ureteral peristalsis. In the re-evaluation, we performed multiple punch biopsies under ureteroscopy and this patient was diagnosed with ureteral cancer. This result suggests that the space occupying lesion of ureter at early stage in the imaging is usually difficult to be identified from the features caused by ureteral peristalsis. Thus, further examination is necessary for FISH-positive patients to avoid misdiagnosis.

Urinary nuclear matrix protein 22 (NMP22), bladder tumor antigen (BTA) and ImmunoCyt have been applied to screen UC for several years, but these methods still have poor sensitivity and specificity (Lamm et al., 2000). According to the findings above, we speculate that FISH is superior to cystoscopy, ureteroscopy and imaging examination in the early diagnosis of UC due to the changes in chromosomes of cancer cells preceding the morphological changes. Another characteristic of FISH is that it can not be influenced by the Bacillus Calmette-Guerin (BCG). O'Donnell et al (2004) treated bladder cancer with BCG for 3 months or longer, and then FISH was performed. Their results revealed that the treatment

did not affect the results of FISH but significantly influenced those of cytology and BTA Stat. Moreover, FISH is a non-invasive method and has no complications, and there are no contradictions for patients receiving FISH. Therefore, patients are compliant to the examination. Thus, we postulate that FISH has important clinical implications in the early diagnosis of UC in asymptomatic hematuria patients.

ACKNOWLEDGEMENT

The study was supported by the Research Funds of the ministry of health (WKJ2007-3-001)

REFERENCES

Halling KC, King W, Sokolova IA, Meyer RG, Burkhardt HM, Halling AC, Cheville JC, Sebo TJ, Ramakumar S, Stewart CS, Pankratz S, O'Kane DJ, Seelig SA, Lieber MM, Jenkins RB (2000). A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. J. Urol., 164(5): 1768-1775.

Lamm DL, Blumenstein BA, Crissman JD, Montie JE, Gottesman JE, Lowe BA, Sarosdy MF, Bohl RD, Grossman HB, Beck TM, Leimert JT, Crawford ED (2000). Maintenance bacillus Calmette-Guerin immunotherapy for recurrent Ta, T1 and carcinoma in situ transitional cell carcinoma of the bladder: a randomized Southwest Oncology Group Study. J. Urol., 163: 1124-1129.

Liu BC, Wang Z, Zhang YF (1999). Etiology and Clinical Strategy of Asymptomatic Microscopic Hematuria. J. Moder. Urol., 4(2): 69.

O'Donnell MA, Lilli K, Leopold C; National Bacillus Calmette-Guerin/Interferon Phase 2 Investigator Group (2004). Interim results from a national multicenter phase II trial of combination bacillus Calmette-Guerin plus interferon alfa-2b for superficial bladder cancer.J. Urol., 172(3): 888-893.

Roy JY, Staerkel GA, Ayala AG (1992). Cytologic and histologic features of superficial bladder cancer. Urol. Clin. North. Am., 19: 435-453.

Sarosdy MF, Schellhammer P, Bokinsky G, Kahn P, Chao R, Yore L, Zadra J, Burzon D, Osher G, Bridge JA, Anderson S, Johansson SL, Lieber M, Soloway M, Flom K (2002). Clinical evaluation of a multi-target fluorescent in situ hybridization assay for detection of bladder cancer. J. Urol., 168: 1950-1954.

Sokolova IA, Halling KC, Jenkins RB, Burkhardt HM, Meyer RG, Seelig SA, King W (2000). The development of a multitarget, multicolor fluorescence in situ hybridization assay for the detection of urothelial carcinoma in urine. J. Mol. Diagn., 2(3): 116-123.

Wang Q, Yi SL (2008). Diagnosis and Staging of bladder cancer: a report of 50 cases. Chin. J. Misdiag., 8(27): 6733-6732.

Wang P, Jin FS, Ye J, Wu G, Li Yf, Zhang Y, Liang P (2009). Chromosome aberration of urine exfoliated cells to diagnose bladder neoplasm by fluorescence in situ hybridization. Acta. Acade. Med. Milit. Tertiae., 31(10): 948-951.