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Efficacy of povidone-iodine against accidental tumor incision during nephron-sparing surgery: experimental study in patients with renal cell carcinoma

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Abstract

Purpose: Accidental tumor incision (ATI) can occur during nephron-sparing surgery (NSS) and correlates with recurrence and metastasis. This study investigated risk factors of intraoperative ATI in renal cell carcinoma (RCC) patients after NSS and the efficacy of povidone-iodine for ATI.

Methods: A retrospective analysis was performed on 150 consecutive stage I (pT1N0M0) RCC patients who underwent NSS at The Second Hospital of Tianjin Medical University between May 2010 and October 2015 for the causes of ATI. Furthermore, *in vitro* experiments investigated whether tumor cells remained on the surface of scissors and the effect of treatment with povidone-iodine on the number of remaining 786-O cells.

Results: Among the 150 cases, 15 showed ATI, of which three suffered local recurrence during a median follow-up of 56 months. Pseudocapsules, satellite nodules, and renal cystic tumors were observed in ATI cases. *In vitro* experiments showed that tumor cells remained on the surface of scissors after ATI during NSS and that 0.5% povidone-iodine effectively killed tumor cells in 30 minutes.

Conclusions: The probability of ATI is high in patients with complex-type RCC during NSS. ATI potentially increases the chance of metastasis and local recurrence, and 0.5% povidone-iodine kills tumor cells more effectively than distilled water.

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Introduction

The clinical application of nephron-sparing surgery (NSS) for treating stage I (pT1N0M0) renal cell carcinoma (RCC) has gradually increased owing to improvements in surgical concepts and the development of diagnostic imaging techniques.[1] NSS with renal preservation has become the gold standard for treating pT1N0M0 RCC because of feasible surgical techniques and improved prognosis.[2–4] Complete tumor resection is a significant surgical principle for any tumor type.[5] A prior study found that the rate of tumor seeding and metastasis can be effectively reduced by avoiding tumor incisions.[6] However, accidental tumor incision (ATI) occasionally occurs in patients treated with NSS and is associated with tumor recurrence and metastasis.[7] To investigate the risk factors for tumor recurrence and metastases associated with ATI, we collected data from pT1N0M0 RCC patients who underwent NSS between 2010 and 2015. Then, causes of ATI and consequences of tumor excision were analyzed in patients who underwent tumor resection. Through *in vitro* experiments, we explored the relationship between tumor excision and tumor recurrence, as well as the effect of povidone-iodine on reducing metastasis and recurrence after the appearance of ATI.

Materials and methods

Patients

This retrospective study enrolled 150 consecutive pT1N0M0 RCC patients who were treated with laparoscopic NSS at The Second Hospital of Tianjin Medical University (Tianjin, China) between May 2010 and October 2015.

Cell culture

786-O cells, which are a cell line derived from a primary clear cell RCC lesion, were provided by Dr. Wang Yong (Institute of Urology, the Second Hospital of Tianjin Medical University, Tianjin, China). Cells were cultured in RPMI-1640 medium containing 10% fetal bovine serum in a 5% CO_2 incubator (HERAcell150, Thermo Fisher Scientific, Rockford, IL, USA) at 37°C with constant humidity.

In vitro experiments

Fresh human RCC samples were incised with laparoscope scissors *in vitro*, and surfaces of the scissors were examined to confirm whether RCC cells remained. The scissors were washed with normal saline and shaken several times, followed by cytologic examination of smears. Normal saline was added on a glass slide, which was fixed with 95% ethanol for 20 minutes and washed three times with phosphate-buffered saline (PBS). Afterwards, the core was stained with hematoxylin (G1120, Solarbio, Beijing, China) for 2 minutes, washed with water for 1 minute, and then cytoplasm was stained with eosin (G1120, Solarbio) for 1 minute and washed with water. The presence of tumor cells was also determined by staining with acridine orange and observing the results under an IX71 microscope (Olympus Optical Co.,

Tokyo, Japan). Smears were fixed with 95% ethanol for 20 minutes, and excess liquid was removed using filter paper. The smears were then acidified with 1% acetic acid for 30 seconds. Slides were stained with 0.01% acridine orange (CA1142, Solarbio) for 30 seconds and washed with PBS. Afterwards, slides were washed with 0.1% HCL in PBS, soaked in 0.1 mol/L $CaCl_2$ solution for 30 seconds, and washed three times with PBS. Finally, the slides were covered with a coverslip and observed and imaged with a fluorescence microscope at 520-nm excitation wavelength.

786-O cells were plated in 24-well plates at a density of 4000 cells/well. The control group was treated with normal saline. The experimental group was divided into the distilled water group and the 0.5% povidone-iodine solution group; both were soaked for either 5 or 10 minutes, and then washed with PBS for 3 minutes. Then RCC

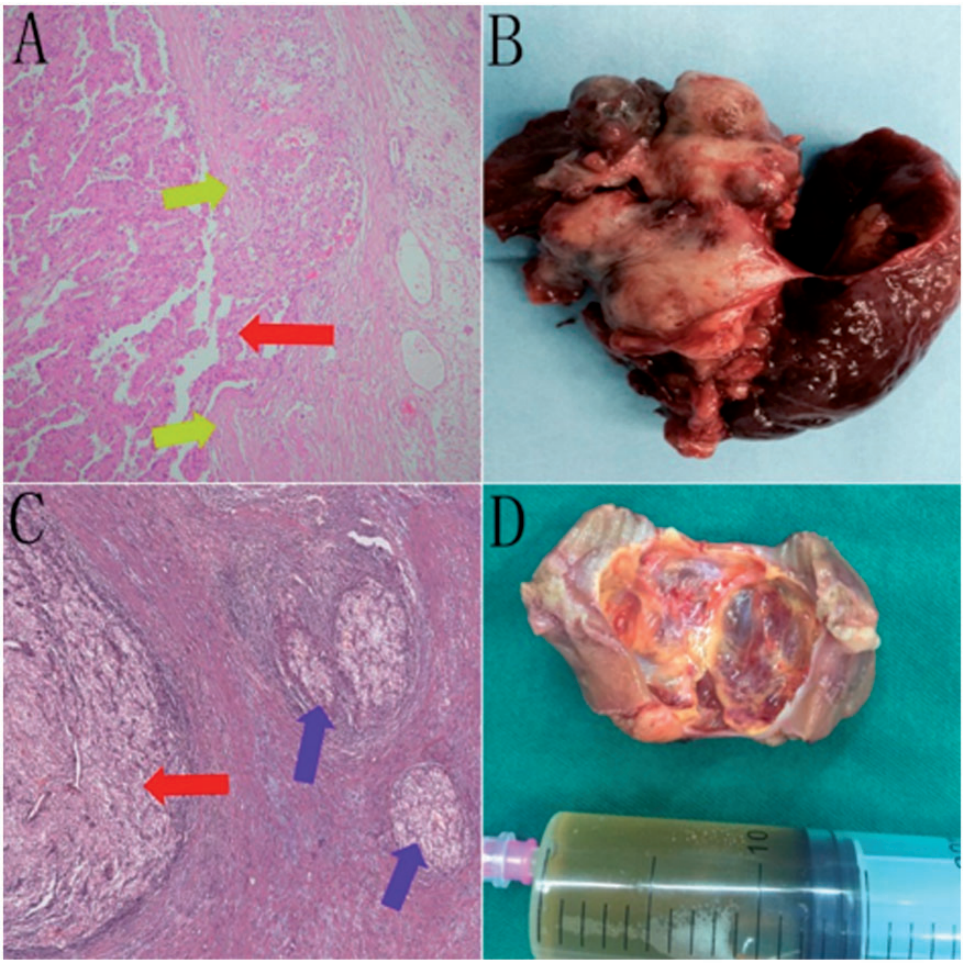

Figure 1. Analysis of risk factor for ATI in 15 pT1N0M0 RCC patients with ATI.
Notes: a, Tumor with pseudocapsule invasion. b, Tumor with irregular growth. c, Tumor with satellite nodules. d, Renal cystic tumor. Red arrow: tumor, yellow arrow: pseudocapsule, blue arrow: satellite nodule. ATI, accidental tumor incision; RCC, renal cell carcinoma.

cells were stained with 10 μg/mL Hoechst 33258 (C0020, Solarbio) for 20 minutes. Cells were washed with PBS, covered with coverslips, and then observed under a FV1000 confocal microscope (Olympus Optical Co.).

Results

Among the 150 cases, the median age was 62.5 years and the average tumor diameter was 3.4 cm (range: 1–7 cm). Fifteen patients showed ATI during surgery, and these cases were followed up for a median duration of 56 months. Among these 15 patients, two had pseudocapsule invasion (Figure 1a), four presented irregular growth tumor (Figure 1b), one exhibited satellite nodules (Figure 1c), two showed renal cystic tumor (Figure 1d), and two showed intraoperative hemorrhage (Table 1).

Tumor samples were also incised using laparoscope scissors *in vitro* and observed using light and fluorescence microscopy. Data from *in vitro* experiments showed that tumor cells remained on the surface of scissors after ATI (Figure 2).

768-O cells were treated with distilled water and 0.5% povidone-iodine for 30 minutes, followed by staining with Hoechst 33258. Hoechst is a nuclear membrane-permeable fluorescent dye. Hoechst staining of normal cell nuclei is round and blue, while the nuclei of dead cells are lobulated and fragmented. As the results showed, 768-O cells were killed in 30 minutes by distilled water, and the morphology of the cells treated with distilled water changed. However, 0.5% povidone-iodine killed 768-O cells more effectively than distilled water treatment (Figure 3).

During follow-up, local recurrence occurred between 3 months and 4 years in three cases. One case underwent seeding metastasectomy, one received radical nephrectomy, and the other was accompanied by multiple lung and liver metastases, and died 4 months after surgery (Table 2). Among the three cases of local recurrence, tumors of one case migrated onto the surface of the kidney (Figure 4a), and tumors of the other two cases metastasized to peripheral and adipose tissues (Figure 4b–d). The four recurrences were found among 135 cases without ATI during follow up, yielding a recurrence rate of 2.96% (Table 3).

Table 1. Type of accidental tumor incision during nephron-sparing surgery.

Characteristics	Value
Sex	
Male	12
Female	3
Tumor diameter (cm)	
<4.0	11
4.0–7.0	4
Peritumoral pseudocapsule	
Absent	4
Present	2
Incision tumor	4
Satellite nodules	1
Renal cystic tumor	2
Intraoperative hemorrhage	2

Discussion

In the genitourinary system, renal cancer is the third most common malignancy, accounting for 4% to 5% of all male malignancies and 2% to 3% of all female malignancies in the United States.[8,9] Approximately 63,990 cases of renal masses are expected to be diagnosed *in situ*, among which approximately 14,400 *in situ* cases are expected to lead to death.[10] With the recent development of diagnostic imaging technologies, there have been great advances in the detection of early asymptomatic pT1N0M0 RCC. Owing to improvements in surgical concepts and the technical level of surgeons, the clinical application of operation-based interventions in renal units has gradually

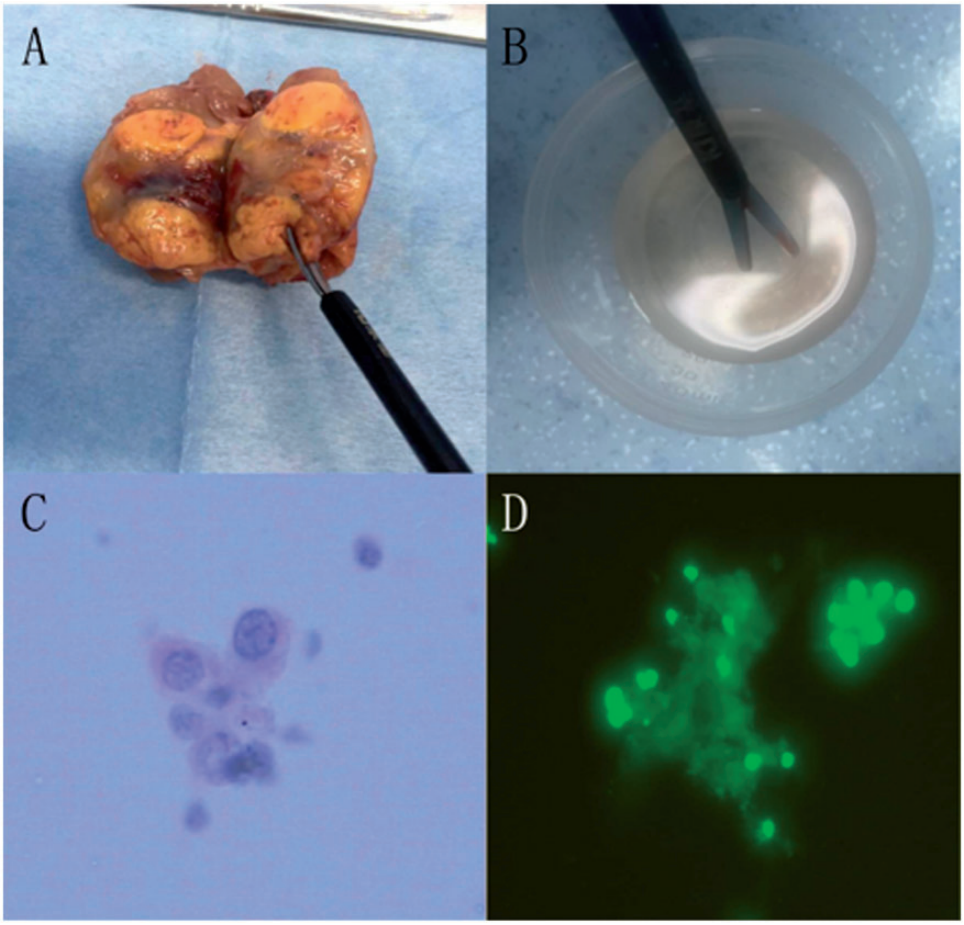

Figure 2. Tumor cells remaining on the surface of scissors after ATI. ATI, accidental tumor incision
Notes: a, Human RCC samples are incised with laparoscope scissors *in vitro*. b, Scissors were dipped into the medium and shaken several times. c, Hematoxylin–eosin staining showing that tumor cells remained on the surface of the scissors. d, Acridine orange staining showing that tumor cells remained on the surface of scissors. RCC, renal cell carcinoma.

increased.[11–13] Additionally, NSS is increasingly used in the treatment of pT1N0M0 RCC.[14] Nevertheless, ATI occurs more prevalently as the use of NSS increases.

Although *in vivo* experiments have indicated that the possibility of metastasis and recurrence can be reduced with water or antiseptic lavage, basic research has shown that tumor metastasis and recurrence are unavoidable after ATI.[15,16] However, according to prior studies, it is still controversial whether ATI increases the incidence of metastasis or recurrence.[17–19] Another study showed that, contrary to consensus, local recurrence is more common in patients with normal surgery than in patients with ATI.[9] However, such reports may not fully represent the impact of ATI on local recurrence owing to fewer cases of ATI. Currently, there are only two reports about ATI, neither of which investigated the reasons for ATI.

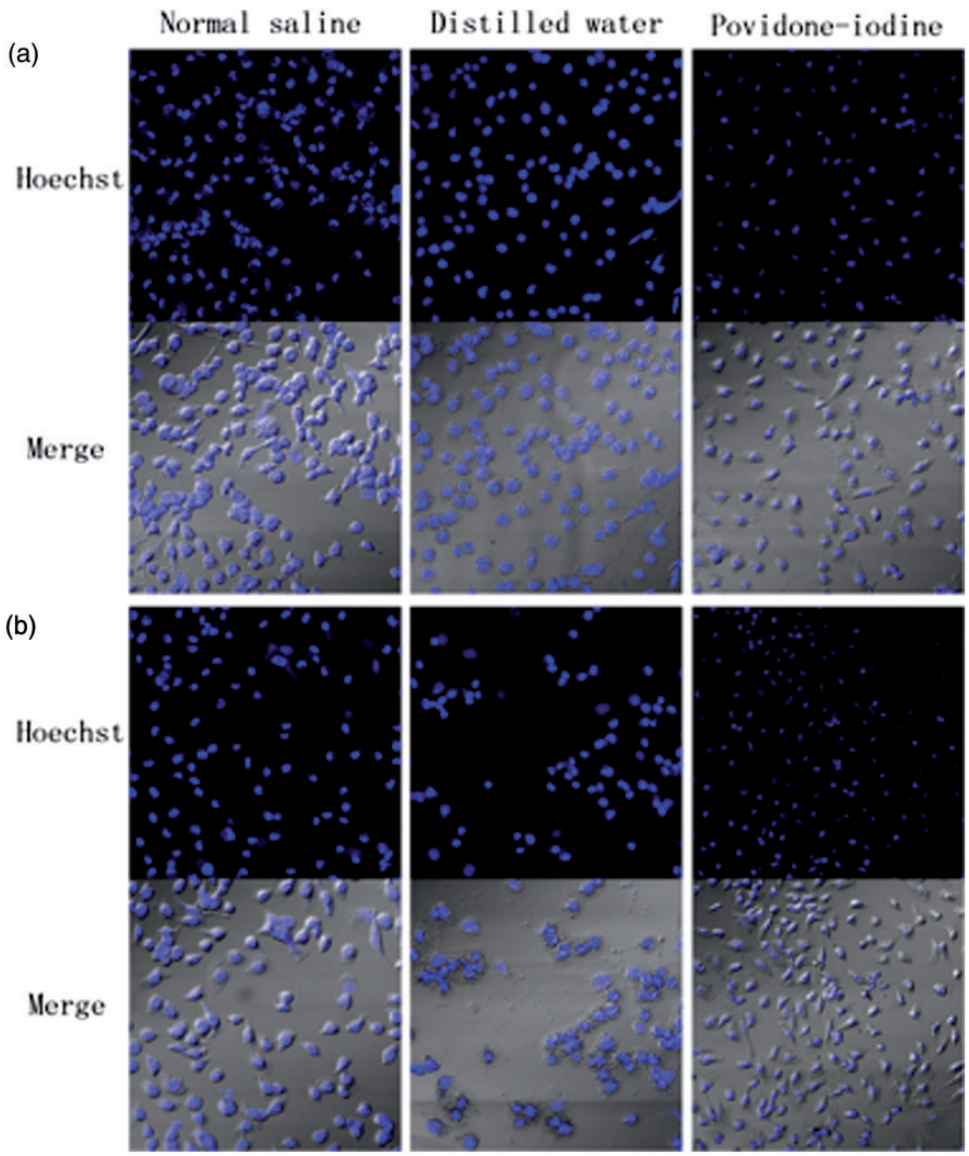

Figure 3. The effect of distilled water and povidone-iodine on 786-O cells.
Notes: 786-O cells were treated with normal saline (control), distilled water, and 0.5% povidone-iodine in 24-well plates and observed under a confocal microscope. a, Treatment for 5 minutes. b, Treatment for 10 minutes.

To reduce the risk of tumor recurrence and metastasis, we analyzed the reason for ATI in patients undergoing NSS and impact of tumor incision. Among 150 cases receiving NSS, ATI occurred in 15 cases. Our analysis found that the risk factors for ATI included pseudocapsule invasion, irregular tumor growth, satellite nodules, and renal cystic tumors. Meanwhile, intraoperative hemorrhage may interfere with the operator's judgment.

Table 2. Analysis of metastasis and local recurrence in patients with ATI.

Reason for ATI	Recurrence (n)	Type of recurrence
Invading pseudocapsule	0	–
Irregular growth	1	tumors migrated on the surface of the kidney
Satellite nodules	1	peripheral adipose tissue metastases
Renal cystoma	1	peripheral adipose tissue metastases
Intraoperative hemorrhage	0	–

Note: ATI: Accidental tumor incision.

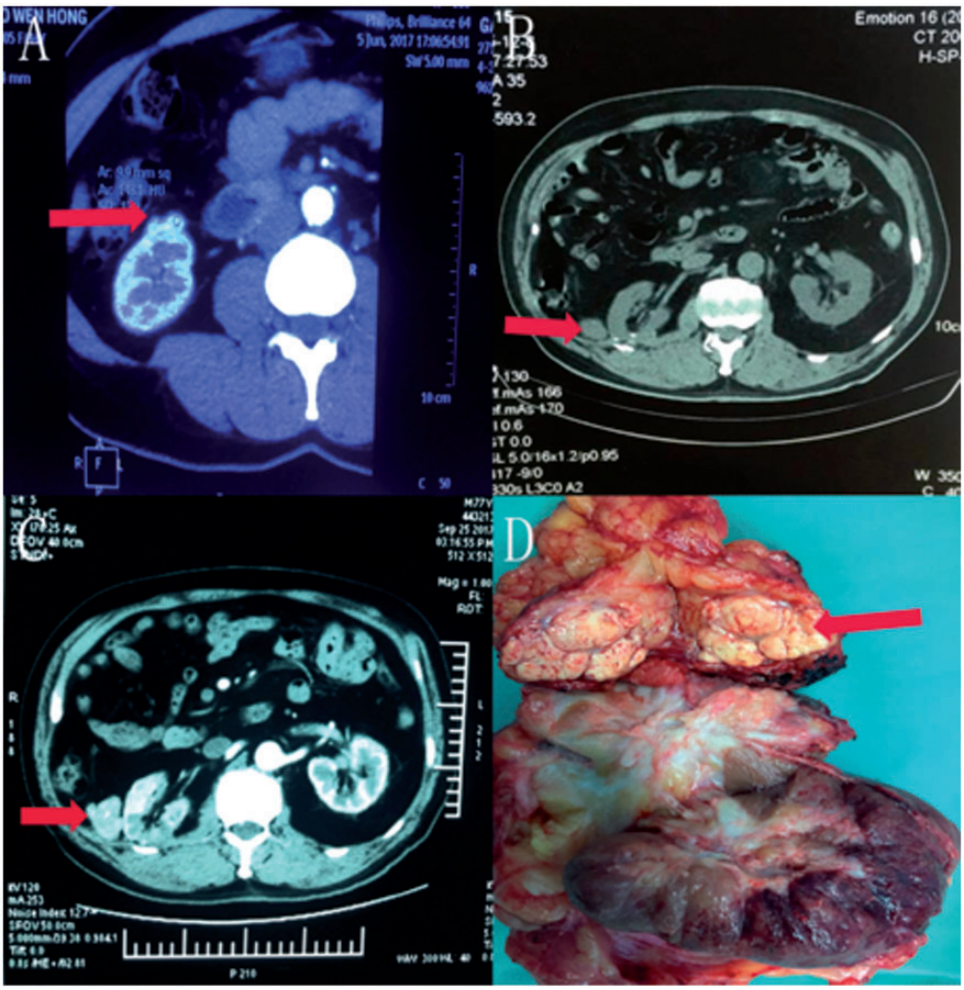

Figure 4. Analysis of metastasis in three pT1N0M0 RCC patients with local recurrence after ATI.
Notes: a, Implantation metastasis on the surface of the kidney. b, c and d, Metastasis of tissues surrounding the kidney. Red arrow: metastatic tumor. RCC, renal cell carcinoma; ATI, accidental tumor incision.

Table 3. Pathological classifications and relapse characteristics.

	Non-ATI				ATI				
Pathological type	Number of patients	Median follow-up time (months)	Relapse number	Relapse rate (%)	Number of patients	Median follow-up time (months)	Relapse number	Relapse rate (%)	P-value
Clear cell cancer	121	56	4	33.01	14	56	3	21.42	0.037
Papillary cell cancer	12	56	0	0	1	56	0	0	–
Chromophobe	2	56	0	0	0	56	0	0	–

Note: ATI: Accidental tumor incision.

A previous study concluded that 82% of RCC cases will develop pseudocapsules.[8] Pseudocapsules are an easily identifiable marker of tumor-substantial demarcation,[20] and can benefit surgeons in their efforts to efficiently remove complete tumors, especially in blunt tumor enucleation.[21] According to our research, some tumors might grow invasively and invade the pseudocapsule. There are two possible situations for this: on the one hand, the pseudocapsule is incomplete and tumor is incised; on the other hand, the pseudocapsule is incomplete, but the tumor is completely contained within the pseudocapsule when it is removed. The former can be considered ATI and have the possibility of metastasis and recurrence, while the latter is almost impossible. Satellite nodules were another reason for ATI. Because pT1N0M0 RCC is <7 cm, most satellite nodules are only found by pathological examination after surgery. Meanwhile, renal cystic tumor was also a risk factor of ATI because the morphology of cystic tumors is similar to renal cysts. The main pathology types of renal cystic tumors are low malignant potential clear cell carcinoma. Clinicians should distinguish between tumors and cysts by careful preoperative diagnosis to avoid the occurrence of ATI.

Based on our cases, local recurrence appeared in three cases among those with ATI. Tumors of one of these cases migrated onto the surface of the kidney, and tumors of the other two cases had metastases to peripheral and adipose tissues.

To decrease the risk of tumor seeding and metastasis after ATI, we should treat or replace equipment. A prior study suggested that tumor cells can be killed after ATI by treatment with electricity.[22] Currently, tissues around solid tumors are often sealed with electrocoagulation after ATI. However, distilled water is always used during ATI treatment in renal cystic tumors. Our study showed that 0.5% povidone-iodine had a more lethal effect on RCC cells than distilled water over a short time period.

This study was a retrospective single-center empirical study with some deficiencies. The cases enrolled were performed by different surgeons. Hence, there were differences in the surgical skills among these surgeons. The induction of risk factors for ATI is very informative and instructive; however, because of the limited number of cases and no statistical analysis, multi-center peer reviews are needed to test the results obtained from our study for the representativeness and reliability of the conclusions. Additionally, we simply verified the possibility of recurrence after ATI by *in vitro* experiments and found that 0.5% povidone-iodine killed tumor cells more

effectively than distilled water. Recurrence could be reduced by effective treatment of antiseptic lavage, but we did not prove this effectiveness in patients. Therefore, clinical validation is necessary in the next stage.

Conclusions

Our data elucidated the impact of ATI on tumor metastasis and local recurrence in RCC patients undergoing NSS as well as the risk factors for ATI, including pseudocapsules, satellite nodules, irregular tumor growth, and renal cystic tumors. The probability of ATI is high in partial nephrectomy for these types of RCC. In accordance with previous experiments in the literature, our study suggested that tumor cells could remain on the surface of scissors and have the ability to seed or recur after ATI. Additionally, 0.5% povidone-iodine killed more RCC cells than distilled water in 30 minutes. Povidone-iodine treatment may decrease potential implant metastasis or recurrence of non-solid tumors after ATI. In summary, it is particularly critical that ATI be avoided by preoperative diagnosis and assessments of tumor shape.

Abbreviations

Accidental tumor incision (ATI); renal cell carcinoma (RCC); nephron-sparing surgery (NSS)

Ethics approval and informed consent

This study was approved by The Second Hospital of Tianjin Medical University Ethics Committee and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of subjects under study have been omitted.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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