

Peer Review File

Article Information: Available at <http://dx.doi.org/10.21037/tau-20-948>

Response to Reviewer:

Comment 1: English language of the paper is poor.

Reply 1: We thank the reviewer for the constructive comment. The manuscript has been further polished by different co-authors and native speakers to ensure accuracy and clarity. All the changes in the manuscript are highlighted with a yellow background.

Comment 2: Line 22-24, page 1, variables collected are described in a confused way. The authors should consider to describe candidate risk factors and outcomes separately. Multivariate seems incorrect, please consult biostatistician.

Reply 2: We would like to express our sincere thanks to the reviewer for this constructive comment. We have removed the description of the variable collection in this paragraph and described the variables in the methods section. Due to the rarity of the disease, there is no literature with sufficient sample size focused on risk factors for the postoperative recurrence rate of KSM. Our recent study found that in another bladder disease, urinary tract infection, long-term indwelling catheter, urinary calculus, squamous metaplasia, and atypical hyperplasia are independent risk factors for recurrence of glandular cystitis (CG). So we referred to this paper before we include appropriate variables. We described candidate risk factors and outcomes separately in Methods, Page 3 Line 23-31, and Page 4 Line 1-5. We consulted two experienced statisticians and conducted multivariate analysis under their guidance, and the results were the same as before. Please check the details in the revised manuscript.

Changes in the text: **Page 1 Line 23-28; Page 3 Line 23-31; Page 4 Line 1-5**

Original version: Methods: The clinical data of 257 patients with KSM who underwent transurethral resection in Xiangya Hospital were retrospectively collected. Follow-up data were available for 223 patients. Possible risk factors including patient's age; smoking status, course of the disease, urinary tract infection, lower urinary tract obstruction, leukoplakia size and location, surgical margin, and pathological results

were retrospectively analyzed by univariate and multivariate analyses with Cox regression.

Revised version: Methods: The clinical data of 257 patients diagnosed with KSM who underwent transurethral resection in Xiangya Hospital from January 2010 to November 2018 were retrospectively collected. Follow-up data were available for 223 patients. To identify the risk factors for recurrence, we performed univariate and multivariate cox regression analysis successively. The correlation between KSM and bladder cancer was investigated from follow-up data.

Data collection

Epidemiological data obtained from the electronic medical record system included clinical characteristics (sex, age, smoking status, main symptoms, and urologic history details), as well as leukoplakia and treatment-related data (leukoplakia location, leukoplakia size, histopathological characteristics, the extent of resection, recurrence and follow-up period) were analyzed retrospectively. Urologic history details, including the history of urinary infection, lower urinary tract obstructions and disease course of KSM (from the first symptom(s) until surgical management), were collected.

Study outcomes

The primary outcome was to identify the risk factors of KSM recurrence. Secondary outcomes were to explore the correlation between KSM and bladder cancer. Relapse was defined as the occurrence of cystoscopy that revealed primary lesions or neoplasms and a biopsy that confirmed keratinizing squamous metaplasia. Lesions' histological type was evaluated according to the most updated 2016 WHO classification criteria (9).

Comment 3: Results of abstract. In general, HRs and their 95%CIs should be reported here.

Reply 3: Thank you very much for your constructive comment. We have reported HRs and their 95%CIs in the results of the abstract. Please check the details in the revised manuscript.

Changes in the text: Page 2 Line 1-6

Original version: Univariate Cox analysis indicated that postoperative urinary tract infection and pathological bladder atypical urothelial hyperplasia were significant recurrence factors. Multivariate Cox analysis suggested that pathological bladder

atypical urothelial hyperplasia was the independent prognostic factor for recurrence.

Revised version: Univariate Cox analysis indicated that urinary tract infection [hazard ratio (HR)=2.111, 95% confidence interval (CI): (1.043-4.271); P=0.038], and atypical urothelial hyperplasia of the bladder (HR=4.191, 95%CI: 2.006-8.756; P<0.001) were significant recurrence factors. Multivariate Cox analysis suggested that atypical urothelial hyperplasia of the bladder (HR=3.506, 95%CI: 1.663-7.392; P=0.001) was the independent risk factor for recurrence.

Comment 4: The introduction of this study is written very poor, too much unrelated information. The study focus is predictors of reoccurrence, so the authors should briefly review existing literature on this topic, as well as the clinical significance of this research topic.

Reply 4: Thank you very much for your constructive comment. We conducted an extensive search of the existing literature on Keratinizing squamous metaplasia (KSM) of the bladder and found no literature on the recurrence of the disease. And we rewritten the introduction, removed any irrelevant parts of the topic. In the introduction, we briefly introduced the incidence, pathological features, clinical features and treatment of this disease. Transurethral resection (TUR) is the standard treatment, but some patients suffer from KSM recurrence. So we identified the risk factors for recurrence and attempted to explore the relationship between KSM and bladder cancer. Please check the details in the revised manuscript.

Changes in the text: Page 2 Line 15-31; Page 3 Line 1-9

Original version: Keratinizing squamous metaplasia (KSM) of the bladder is also known as vesical leukoplakia or keratinizing cystitis of the bladder. It consists of the replacement of the normal bladder urothelium with a keratinizing stratified squamous epithelium(1). Rokitansky et al. first described the pathological features of KSM in 1862(2). It is a rare disease, whose incidence has been reported to be 1:10,000 of hospital admissions to the Mayo Clinic(2,3). With the improvement of cystoscopy and pathological examination technology, the incidence of such cases tends to increase in recent years(4). The pathogenesis of KSM remains incompletely understood, but its development is related to multiple different factors. Clinically, it was found that the disease was closely related to lower urinary tract infection, obstruction, and proliferative lesions, chronic cystitis, long-term indwelling catheters, etc.(2,5).

Therefore, it was believed that vesical leukoplakia might be caused by chronic irritation or inflammation leading to squamous metaplasia. Urothelium has a great metaplasia and proliferation ability, by chronic infection and other stimulation, it replaced by stratified squamous epithelium(6).

The clinical manifestations of vesical leukoplakia are mainly bladder stimulation symptoms, such as frequency of urination, urgency, urination pain, and hematuria or urinary tract obstruction(4). Its diagnosis depends on cystoscopy and histopathology. During the cystoscopy, when water was injected into the bladder, a large number of exfoliated epithelial and keratin fragments were seen floating in the water, the phenomenon of "snowstorm" appeared. The lesion showed gray and white irregular patchy changes, slightly higher than the normal mucosal plane, the edge is clear(7). Clinical observation showed that after the plaque was removed, the underlying tissue was prone to bleeding(2,5). Benson et al. reported a 21% incidence of bladder cancer in 78 patients with keratinized bladder metaplasia(8). It is now generally considered a pre-malignant lesion. The clinical statistics of its malignant transformation rate is 15%~20%, there are reports as high as 28%, the vast majority of which is squamous cell carcinoma(9). Although different therapies are reported in the literature to manage KSM, due to the risk of bladder cancer development from KSM, transurethral resection (TUR) or fulguration for the total removal of the affected mucosa and close follow-up were widely used as treatment strategies(4,10).

Patients' quality of life improved after these treatments, as both urinary symptoms and the frequency of urinary tract infections (UTI) improved(11). Also, because of the histological regression of the lesion, the risk of bladder cancer is reduced(12). Our recent study found that in another bladder disease, urinary tract infection, long-term indwelling catheter, urinary calculus, squamous metaplasia, and atypical hyperplasia are independent risk factors for recurrence of glandular cystitis (CG)(13). Besides, we evaluated differentially expressed mRNAs and lncRNAs in glandular cystitis(14). The etiology of KSM is similar to that of CG, both of which are due to long-term stimulation, inducing the transitional epithelium of the bladder to different epithelium. There are many cases of vesical leukoplakia in China. However, there are currently no studies on the risk factors for the recurrence of patients with vesical leukoplakia. Therefore, this study aims to retrospectively analyze potential recurrence factors in Chinese patients with vesical leukoplakia after surgical management in Xiangya Hospital. We present

the following article in accordance with the STROBE reporting checklist.

Revised version: Keratinizing squamous metaplasia (KSM) of the bladder is also known as vesical leukoplakia or keratinizing cystitis of the bladder. KSM is a rare disease with a reported incidence of 1:10,000 (1, 2). With the improvement of cystoscopy and pathological examination technology, such cases' incidence tends to increase in recent years(3). Pathologically, it consists of replacing the normal bladder urothelium with a keratinizing stratified squamous epithelium(4). The clinical manifestations of vesical leukoplakia are mainly bladder stimulation symptoms, such as frequent urination, urgency, urination pain, hematuria and urinary tract obstruction (3).

The pathogenesis of KSM remains incompletely understood. Clinically, it was found that the disease was closely related to a long-standing stimulus such as lower urinary tract infection, obstruction, proliferative lesions, chronic cystitis and long-term indwelling catheters (2, 5). Our recent study found that in another bladder disease, urinary tract infection, long-term indwelling catheter, urinary calculus, squamous metaplasia, and atypical hyperplasia are independent risk factors for glandular cystitis recurrence (CG)(6). KSM is now generally considered a pre-malignant lesion. Several previous case reports showed that the clinical statistics of its malignant transformation rate is 15%~20% (7). Transurethral resection (TUR) or fulguration for the total removal of the affected mucosa and close follow-up were widely used as treatment strategies (3, 8); however, some patients suffer from KSM recurrence after receiving surgical management. Because of the disease's rarity, there is little literature with enough sample size focused on risk factors for postoperative recurrence rate of KSM. This study aims to retrospectively analyze potential recurrence factors in patients with KSM after surgical management in a single center with 223 patients, and explore the correlation between KSM and bladder cancer. We present the following article in accordance with the STROBE reporting checklist.

Comment 5: Methods. The authors should use a separated paragraph to describe how covariates and outcomes were collected.

Reply 5: We would like to express our sincere thanks to the reviewer for this constructive comment. We used two paragraphs to describe the collection of variables

and the results of the study. Variables included clinical characteristics and treatment-related data. The outcomes were in two parts: the primary outcome was to identify risk factors of KSM recurrence. Secondary outcomes were to investigate the correlation between KSM and bladder cancer. Please check the details in the revised manuscript.

Changes in the text: Page 3 Line 23-31; Page 4 Line 1-5

Original version: In the early stage, we searched the digital electronic medical record management database of our hospital, the patient's data have been secured. By using the search keywords “keratinizing squamous metaplasia” or “vesical leukoplakia” or “leukoplakia of bladder”. The information retrieved included demographic, chief complaints, history of present illness, prior medical history, physical examination, laboratory examination, and cystoscopy, histopathological reports, and operative data. Possible risk factors for recurrence of KSM include patient’s sex, age, smoking status, course of the disease(from the first symptom(s) until surgical management), urinary tract infection or not, history of urinary calculi, lower urinary tract obstruction or not, leukoplakia size and location, surgical margin, and whether there was atypical urothelial hyperplasia in pathological results(4). Urinary tract infection was evaluated by urine routine and positive urinary cultures. Lower urinary tract obstruction included bladder neck contracture, urethral stricture, urethral caruncle, urethral calculus, and neurogenic bladder, etc., which were diagnosed with cystourethroscopy and urodynamic outcomes. Lesions histological type was evaluated according to the most updated 2016 WHO classification criteria. (15).

Revised version:

Data collection

Epidemiological data obtained from the electronic medical record system included clinical characteristics (sex, age, smoking status, main symptoms, and urologic history details), as well as leukoplakia and treatment-related data (leukoplakia location, leukoplakia size, histopathological characteristics, the extent of resection, recurrence and follow-up period) were analyzed retrospectively. Urologic history details, including the history of urinary infection, lower urinary tract obstructions and disease course of KSM (from the first symptom(s) until surgical management), were collected.

Study outcomes

The primary outcome was to identify the risk factors of KSM recurrence. Secondary outcomes were to explore the correlation between KSM and bladder cancer. Relapse

was defined as the occurrence of cystoscopy that revealed primary lesions or neoplasms and a biopsy that confirmed keratinizing squamous metaplasia. Lesions' histological type was evaluated according to the most updated 2016 WHO classification criteria (9).

Comment 6: A major limitation of the study is no assessment of treatment-related factors. It seems not reasonable to find only a predictor of reoccurrence.

Reply 6: We would like to express our sincere thanks to the reviewer for this constructive comment. We conducted an extensive search of the existing literature on Keratinizing squamous metaplasia (KSM) of the bladder. Treatment-related factors include the following points: Firstly, the treatment is divided into drug treatment and surgical management. As for drug treatment, there is no conclusion yet, and this research only discussed surgical treatment. Secondly, the surgical method. The patients in this research all adopted the standard treatment method - transurethral resection (TUR). Thirdly, the depth and extent of resection. The resection depth was all to the mucosa and submucosa, and the resection extent was the bladder leukoplakia and the surrounding bladder mucosa ($>2\text{cm}$ or $\leq 2\text{cm}$). We consulted two experienced statisticians. After univariate and multivariate Cox analysis, we only concluded that atypical urothelial hyperplasia of the bladder was the independent risk factor in patients with KSM recurrence. Please check the details in the revised manuscript.