Medical Research Archives

European Society of Medicine

OPEN ACCESS

Published: December 31, 2023

Citation: Abdsessater M, Boustany J, et al., 2023. Urinary Cotinine: A Promising Marker of Recurrence of Non-muscle Invasive Bladder Tumor, Medical Research Archives, [online] 11(12). https://doi.org/10.18103/mra.v11i12.4779

DOI
https://doi.org/10.18103/mra.v11i12.4779

ISSN: 2375-1924

RESEARCH ARTICLE

Urinary Cotinine: A Promising Marker of Recurrence of Non-muscle Invasive Bladder Tumor

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ABSTRACT

Introduction and objectives: Nicotine is well known to be an addictive compound of tobacco, but it is not primarily carcinogenic. It is metabolized to cotinine, which is considered the best marker of tobacco exposure. Little is known whether recurrence rate of bladder cancer is related to nicotine metabolites eliminated in urine. We aim by this study to evaluate the relation between urinary cotinine level and the recurrence of bladder cancer.

Materials and Methods: Between January 2018 and June 2022, a cross sectional study was conducted. Enrolled patients were smokers already diagnosed with non-muscle invasive bladder cancer, treated by resection only, and presenting for a follow up cystoscopy. Included patients had unchanged smoking habits in the day before the procedure, same ethnicity (Caucasians) and no additional professional exposure. Cotinine level was measured on a sample of voided urine before cystoscopy. Tumor recurrence was considered positive independently from the histological type, and was assessed by direct vision cystoscopy. Patients were considered moderate or heavy smokers depending on the level of cotinine in their urine samples (< or > 550 ng/ml respectively). A Fisher Exact Test was used to assess the relationship between variables.

Results: A total of 135 patients was included. Mean age was 64 years (range 36 to 78). The mean duration of smoking was 30.3 years. Urinary cotinine level was > 550 ng/ml in 80 patients (59.26%) and < 550 ng/ml in 55 patients (40.74%). Recurrence was identified in 70 patients (51.85%) and was absent in the remaining 65 (48.15%). Recurrence was observed in 68.75% of the heavy smokers and in 27.27 % of moderate smokers. Cotinine level higher than 550 ng/mL was linked to an increased risk of bladder cancer, with a relative risk of 4.16 (p-value < 0,025).

Conclusion: High urinary cotinine levels (>550 ng/ml) conveys a 4 folds' risk for the recurrence of bladder cancer in smokers. Additional prospective studies are needed to better understand the relation between urinary cotinine levels and bladder cancer, and its usefulness in bladder cancer surveillance.

Keywords: Bladder Cancer, Non-muscle invasive bladder cancer, Urinary Cotinine, Smoking, Tobacco, Tobacco smoke exposure, Recurrence, biomarker

1. Introduction

Bladder cancer is ranked fourth in the list of most common malignancies in men [1]. The non-muscle invasive bladder cancer (NMIBC) sub-type makes up to 70-80 percent of all urothelial bladder tumors, which is in its turn the most prevalent type of bladder neoplasm in the developed world. NMIBC consists of T_a in 70% of cases, T_1 in 20% of cases, and primary Tis in 10% of cases [2].

Among bladder cancer sub-types, NMIBC is known to have a good survival prognosis but a high intravesical recurrence rate. 10-30 percent of recurrences will progress to advanced disease stages [3,4], thus surveillance and early recognition of cancer recurrences are of utmost importance in patient management [5].

Nowadays, cystoscopy is required for finding and diagnosing bladder tumors. It is recommended every 3 to 6 months in association with urine cytology. Being an unpleasant, invasive, and costly procedure, the need for an alternative tool for bladder cancer detection is highly needed.

On the other hand, urine cytology, a harmless test, to detect and monitor urothelial bladder carcinoma, remains unreliable. Despite urine cytology's high specificity, its sensitivity remains very low, leading to false-negative results even in high-grade muscle-invasive disease[6]. Interobserver variability proved that urinary cytology is a pathologist experience-depended test [7]. This fact forces patients to undertake additional procedures and highlights the need for a non-invasive reliable biomarker test.

Many urine-based tests are currently accessible for medical professionals and have gained the approval of the United States Food and Drug Administration (FDA) [8], but none of them are used in clinical practice nor included in the guidelines of hematuria and bladder cancer workup. Until now, these biomarkers are used in the surveillance setting in addition to the classic recommended workup.

After a trans-urethral resection of bladder tumor (TURBT), two scoring systems are available for the prediction of recurrence of NMIBC, according to two models: the EORTC and the CUETO [9].

Tobacco smoking is considered to be one of the principal causes of developing bladder cancer, NMIBC in particular, as it contains around sixty carcinogenic molecules. Nicotine, an addictive compound present in tobacco, is not known to be primarily carcinogenic. All tobacco products contain nicotine. The average concentration of the absorbed nicotine per cigarette smoked lies around one milligram (mg) of the already six to twelve milligrams of nicotine present in the tobacco for every manufactured cigarette [10].

Our literature review highlighted the lack of a correlation between nicotine metabolites excreted in the urine and the recurrence rate of NMIBC in smokers as well as in non-smokers.

Cotinine is the main metabolite of nicotine and is considered the best marker of an individual's tobacco exposure index. 70 to 80% of Nicotine is metabolized in the liver into cotinine CYP2A6 and up to 5% into nornicotine by CYP2A6 and CYP2B6 [11].

This study aims to identify a correlation between urinary cotinine levels (UCL) and the recurrence rate of NMIBC in a dose-dependent relationship.

2. Methods

2.1 DESIGN:

We conducted a prospective cohort study at our university hospital between January 2017 and June 2022.

A sample of 135 patients diagnosed with NMIBC and treated exclusively by transurethral bladder tumor resection was included in our study. During a follow-up visit 3 months after enrollment, these patients answered a questionnaire about their smoking habits and provided a urine specimen for cotinine dosage. A control cystoscopy was done to assess the possibility of tumor recurrence. The institutional review board and ethics committee of the Holy Spirit University of Kaslik - Lebanon (USEK) have approved this prospective cohort study, and a written informed consent was obtained from each enrolled subject.

2.2 PATIENTS:

All patients included in this study, smokers and non-smokers, had an NMIBC treated by transurethral bladder tumor resection, without any adjuvant intravesical therapy (whether a BCG or Mitomycin course). No ethnic differences were present between patients.

The non-inclusion criteria were:

- All patients diagnosed with Muscle Invasive Bladder Cancer (MIBC) or urinary tract cancer, other than NMIBC.
- Patients that received adjuvant systemic or local chemotherapy post TURBT.
- Patients diagnosed having other types of malignancies.
- Patients known to have a history of prior pelvic Radiotherapy.

- Patients with arsenic exposure (professional exposure or in an arsenic-based chemotherapeutic agent).
- Patients with known Schistosoma Haematobium infection or recent history of travel to Egypt.
- Patients with chronic urinary tract infections.

2.3 ASSESSMENTS:

In addition to in-person surveys drawing information on smoking habits, and active or passive smoking exposure (Figure 1), we collected 7 mL from a single void urine sample by a non-invasive procedure from every patient before cystoscopy. Urine samples were collected in red-capped tubes containing no additives and subsequently stored in a -20 °C temperature regulated fridge. These tubes were transported to the laboratory, within 2 days of sample collection, for UCL testing using high-performance liquid chromatography (HPLC).

Q #	Question
1-	How old are you?
2-	What is your gender?
3-	What is your profession?
4-	Have you ever smoked?
4a-	For how long?
5-	Are you a current active smoker?
5a-	What type of smoking products do you use?
5b-	How many cigarettes do you smoke per day?
5c-	Are there any changes in your smoking habits during the past three months? (Decrease/Increase in smoking frequency)
6-	How many cigarettes did you smoke during the past 4 days
7-	When was your last smoked cigarette?
8-	Did you try to quit smoking after your first TURBT?
9-	Did you ever use any nicotine replacement therapy?
10-	Are you aware of the harms caused by tobacco on the prognosis of your bladder cancer?
11-	Are you a subject of a passive smoking exposure at work?
12-	Are you a subject of a passive smoking exposure at home?

Figure 1: Questionnaire on smoking habits

During cystoscopies, the physician inspected the bladder for any apparent recurrent exophytic or flat tumors which were described, counted, measured in size, and resected for further pathology analysis.

We stratified patients into two groups according to the recurrence status of their NMIBC. Every group was further divided into two subgroups according to smoking status. One subgroup contained all 'moderate smokers' where UCL was found to be less than 550 ng/mL and the other subgroup contained the 'heavy smokers' that were found to have a UCL>550 ng/mL (Figure 2).

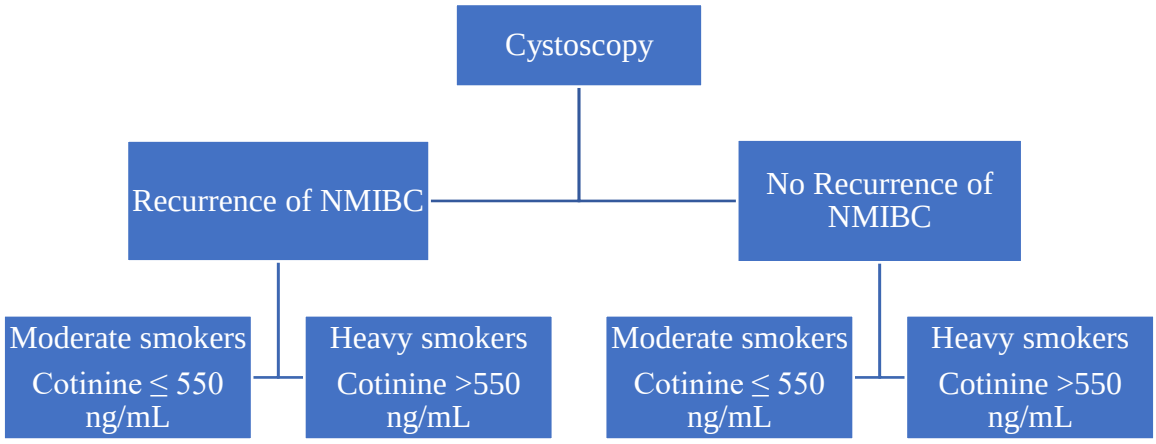

Figure 2: Study design

2.4 STATISTICAL ANALYSIS:

This study aims to establish an association between high UCL and the recurrence of NMIBC. Thus, we used Fisher's Exact test. The following results and their corresponding 95% confidence intervals were calculated using "Statistical Package for the Social Sciences" (SPSS) version 22 as a statistical analysis tool.

3. Results:

In total, 135 patients (92 males and 43 females) were enrolled in this study, aged between 36 and 78 years old (average of 64 years). All patients were Caucasians, with a comparable bladder carcinogens exposure profile. Tobacco intoxication was at an average of 40.4 pack-years. Hence, 135 urine samples were analyzed and measured for their corresponding UCL.

Patients were clustered in a 2 by 2 table with the variables being 'tumor recurrence' and 'no tumor recurrence', in addition to the UCL (Figure 3).

	Recurrence of NMIBC	**No recurrence of NMIBC**	**Total**
Heavy smokers Cotinine > 550 ng/mL	55 (40.74%)	25 (18.51%)	80 (59.26%)
Moderate smokers Cotinine ≤ 550 ng/mL	15 (11.11%)	40 (29.63%)	55 (40.74%)
Total	70 (51.85%)	65 (48.14%)	135

Figure 3: Number of recurred cases of NMIBC according to urinary cotinine levels

The mean duration of smoking was 30.3 years. Urinary cotinine level was > 550 ng/ml in 80 patients (59.26%) and < 550 ng/ml in 55 patients (40.74%). Recurrence was identified in 70 patients (51.85%) and was absent in the remaining 65 (48.15%). Recurrence was observed in 68.75% of the heavy smokers and in 27.27 % of moderate smokers. Cotinine level higher than 550 ng/mL was linked to an increased risk of bladder cancer, with a relative risk of 4.16 (p-value < 0,025).

The null hypothesis 'H0: the proportion of NMIBC with UCL > 550ng/ml is less or equal to the proportion of NMIBC with UCL ≤ 550ng/ml', was rejected by Fisher's exact test.

Consequently, these results proved that a UCL of more than 550ng/ml is associated with a higher risk of NMIBC recurrence at a p-value of less than 0.025, and a relative risk (RR) of 4.16.

4. Discussion:

Nicotine's half-life is relatively short, two to three hours, in comparison to cotinine which has an average half-life of seventeen hours. The elimination rate of each molecule from the body is widely influenced by their distinct half-lives. It takes three to four days to eliminate cotinine from the body of the exposed patient. In contrast, nicotine is eliminated from the body in only a matter of hours. With intermittent nicotine exposure such as that occurs with cigarette smoking, cotinine concentration remains relatively unchanged throughout the day and at near-constant values. Moreover, there are no noteworthy dissimilarities in the pharmacokinetics of nicotine and cotinine in both categories, smokers and non-smokers. Cotinine concentration in biological fluids is a quantitative reflection of a person's exposure to nicotine, which is exclusively relative to tobacco exposure in patients that are not using any other nicotine replacement therapy. Short term monitoring by measuring the urine concentration of cotinine is possible due to its relatively long half-life and its lesser fluctuation rate.

This study demonstrates that UCL can be used as a possible marker to predict the recurrence of NMIBC. It is the first study to implicate the UCL in the management of bladder cancer. It confirms that cotinine levels are a reliable tool to assess the amount of exposure to tobacco, especially since all the patients enrolled in this study have denied any change in smoking habits, for the past three months at least. That is why any important fluctuations in the UCL are less likely, remaining somewhat stable due to its relatively long half-life.

In fact, the declared smoking habits in the survey were compatible with the UCL, where active smokers were found to have higher UCL than non-smokers and passive smokers (Figure 4). Active smokers, or 'Heavy smokers', when correlated to UCL, had the highest rate of NMIBC recurrence among all other categories, reaching 38.5%, while non-smokers or 'Moderate smokers' had a mere 12.5% recurrence rate.

Heavy smokers Cotinine > 550 ng/mL	80 (59.26%)	Active smoker	69 (86.25%)
		Passive smoker	11 (13.75%)
		No known tobacco exposure	0 (0%)
Moderate smokers Cotinine ≤ 550 ng/mL	55 (40.74%)	Active smoker	2 (3.65%)
		Passive smoker	28 (50.90%)
		No known tobacco exposure	25 (45.45%)

Figure 4: Number of active, passive and non-smokers in relation to the urinary cotinine level

It is important to note that cotinine excretion after vegetable consumption does not affect the results. Cotinine food concentrations are vastly reduced after removing food skins and cooking in water; furthermore, the amount of cotinine absorbed by the stomach is negligible and 70% is metabolized in the liver [12]. Therefore, the assumption that approximatively all the cotinine found in tested urine samples is from tobacco exposure can be drawn.

The levels of cotinine are higher in active smokers (10–500 ng ml^{-1}) than those of nonsmokers (1–10 ng ml^{-1}) [13]. There is a correlation between urinary cotinine concentration and moderate daily tobacco consumption (between 11 and 19 cigarettes daily) where the urinary cotinine level (UCL) fluctuates between 20 ng/ml and 550 ng/mL. Heavy smokers (that consume 20 or more cigarettes daily) have higher UCL where they tend to peak between 1000 and 8000 ng/mL. When the patient abstains from smoking for two weeks, this concentration decreases to less than 50 ng/mL. In non-smokers, cotinine can accumulate in their urine from passive tobacco exposure for up to 20ng/mL. Non-smokers with no recorded passive exposure have a UCL of less than 5 ng/mL [14].

A cut-off value of UCL of 550 ng/mL was adopted in this study where all patients below this concentration were known to be non-smokers or subjected to light passive smoking exposure whereas patients that have higher values of UCL were labeled as heavy smokers or subjected to higher levels of passive smoking exposure. Biochemical errors are improbable since the method of cotinine determination is very precise.

In a cohort study conducted by F De Waard et al on female patients in 1995, the risk of lung cancer was found to be proportional to the UCL [13]. Hyun-Suk Jung et al proved that UCL predicts nicotine dependence levels in smokers [15]. Besides, this study demonstrates the relationship between UCL, and indirectly, nicotine intake, with the recurrence of NMIBC. High urinary cotinine levels (>550ng/ml) conveys a four-fold risk for the development of NMIBC in smokers and non-smokers. This type of malignancy is an international major health burden due to its high incidence rate and its rapid recurrence. Once the correlation between UCL and the recurrence of NMIBC is elucidated, better management and monitoring parameters can be offered to the bladder cancer patient for a better life expectancy and quality of life.

Our study found a strong association between UCL and the recurrence of NMIBC. The higher UCL was linked to a four-fold increased risk of NMIBC recurrence. This finding is significant because it suggests that UCL could serve as a reliable and accessible tool for assessing the risk of bladder cancer recurrence in both smokers and non-smokers [16]. This novel approach could help in the early detection and management of NMIBC, potentially reducing the need for frequent and invasive cystoscopies.

While our study provides valuable insights into the potential use of UCL as a biomarker for NMIBC, it's important to acknowledge the limitations. The sample size in your study was relatively small, and further research with larger cohorts and longer follow-up periods is necessary to validate the utility of UCL in clinical practice[17]. Additionally, comparing UCL with other approved biomarkers or diagnostic methods, such as urine cytology, could offer a more comprehensive evaluation of UCL's effectiveness.

Understanding the relationship between UCL and NMIBC recurrence has significant clinical implications. Bladder cancer is a major health concern with high recurrence rates, and finding a non-invasive and cost-effective biomarker could improve patient management and quality of life. By using UCL as a predictive tool, healthcare professionals may be able to tailor surveillance and intervention strategies more effectively, reducing the burden on patients and healthcare systems.

The practical aspects of using UCL as a biomarker should also be addressed. Standardizing cutoff values and measurement techniques for UCL is essential for its clinical application. Furthermore, addressing any potential confounding factors or limitations in detecting UCL, such as cotinine excretion due to dietary sources or passive exposure, is crucial for accurate interpretation[18].

5. Conclusion:

The field of biomarkers for bladder tumor detection and surveillance improves rapidly. To date, no bladder tumor biomarker has proved its superiority to classic urine cytology and cystoscopy. In this study, we highlighted the importance of UCL in the surveillance of NMIBC and suggested it as a promising, accessible, non-invasive, low-cost biomarker that can change the management of bladder cancer by reducing the frequency of cystoscopies. Confirming any promising results by larger-scale studies with bigger sample sizes and longer follow-up periods is primordial and necessary before establishing the need for routine UCL testing in clinical practice.

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