Urinary Level of CA19-9 as a Tumor Marker in Urothelial Carcinoma of the Bladder

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Purpose: To diagnose the urothelial carcinoma of the bladder by measuring CA19-9 level in the urine.

Materials and Methods: This study was conducted on 47 patients with histopathologically confirmed urothelial cancer and 50 control subjects. The urinary level of CA19-9 was measured in both groups by enzyme-linked immunosorbent assay after concentration of urine with Bio-Gel dry beads. Urine cytology was also done in both controls and patients.

Results: The mean urinary level of CA19-9 was 194.59 ± 110.56 u/mL in patients and 11.67 ± 8.42 u/mL in controls (P = .0001). The mean urinary level of CA19-9 in patients with low-grade and high-grade bladder cancer was 206.56 ± 114.56 u/mL and 174.80 ± 94.06 u/mL, respectively (P = .56). Urine cytology by Papanicolaou stain was mostly negative.

Conclusion: It can be concluded that CA19-9 may be a useful non-invasive test to diagnose the urothelial carcinoma of the bladder.

Keywords: urinary bladder neoplasms, tumor markers, CA-19-9 Antigen, urinalysis

Urol J. 2011;8:203-8.
www.uj.unrc.ir

INTRODUCTION

Bladder cancer is a major health problem in the world with more than 63 000 new cases predicted in the US yearly.[1] Smoking is the greatest risk factor for urothelial carcinoma and increases risk of developing the disease four-fold compared to non-smokers.[2] Age, diet, occupational chemical exposure to benzidine dye, and painting industry are other risk factors.[3] Bladder cancer is generally more common in men, but the reason behind this gender biasness is unknown.

Early diagnosis of the bladder cancer is very difficult because there is no distinct associated symptom.[4] Hematuria is one of the most common presenting symptom; however, 90% of individuals with hematuria do not have bladder cancer.[5] Furthermore, gross hematuria usually denotes large tumors; hence, most likely correlates with more advanced disease.

Diagnosis of bladder cancer mostly depends on urine cytology and cystoscopy. Cystoscopy has been proven quite successful in surveillance and follow-ups of patients with previously diagnosed bladder cancer. The drawback of cystoscopy is that it is somewhat expensive, invasive, and uncomfortable.[6] Therefore, it is not a suitable tool for lifelong surveillance of patients with bladder cancer.[7]

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Received December 2010
Accepted May 2011

In conjunction with cystoscopy, urine cytopathology helps identify precancerous and cancerous cells in the urine. But urine cytology is mostly positive in high-grade lesions and its sensitivity to detect low-grade bladder cancer is very low.[8,9]

So other than cystoscopy, it is difficult to diagnose the urothelial carcinoma in early stage. In an isolated paper, it has been shown that a reliable tumor marker is a potential diagnostic tool for urothelial carcinoma along with cystoscopy.[10] Carbohydrate antigen (CA) 19-9 marker is 210 kD tumor-associated glycoprotein antigen present as carbohydrate determinant on glycoprotein. CA 19-9 is characterized by monoclonal antibody 1116-NS-19-9 by immunizing BALB/c mice with human colorectal cancer line. This antibody reacts with CA19-9, which has been identified as a sialylated lacto-N-fucopentose II, an oligosaccharide sharing structural features with Lewis blood group substances. This antigen was localized immunohistologically on fetal epithelia of the colon, small intestine, stomach, pancreas, and liver, and very small concentration on adult gastrointestinal tract and lung tissue. Considerable concentration of CA19-9 is also present in the mucin-rich saliva, seminal fluid, gastric juice, amniotic fluid, urine, ovarian cyst fluid, gall bladder, and duodenal secretions.

CA19-9 is neither tumor nor organ-specific. However, the highest diagnostic sensitivity (85%) and specificity (95%) of CA19-9 are reported for adenocarcinoma of the pancreas. Sensitivity of 70% has been observed in cholangiocarcinomas and gallbladder carcinomas. Very low sensitivity has been reported for the colorectral, stomach, primary liver, bronchial, mucinous ovarian, uterus, and breast carcinomas.[11,12]

The CA19-9 concentration correlated well with the clinical response to treatment. In addition to its use as a diagnostic tool, CA19-9 appears to be a promising marker that can predict recurrence of tumor after pancreatectomy prior to clinical or radiographic evidence of disease relapse.[11,12]

The aim of this study was to determine urinary level of CA19-9 in different stages of bladder cancer and its role as a non-invasive diagnostic tool in low-grade cases of the bladder tumors.

MATERIALS AND METHODS

This case-control study was conducted in the Biochemistry, Urology, and Pathology departments of I.P.G.M.E & R over a period of 18 months and was approved by the ethics committee of I.P.G.M.E & R.

The cases and controls were selected from urology outpatient department and indoor patients. Forty-seven patients were selected from those with histopathologically confirmed bladder carcinoma. Fifty controls were selected from those who had no previous history of any urological disorders.

Patients with hematuria, any age, and any gender were included in the study. Abdominal mass, history of prostatism, urinary tract infection, anorexia, weight loss, or proteinuria were considered as exclusion criteria. A written informed consent was then obtained from each remained participant.

Reagents

-Enzyme-linked immunosorbent assay (ELISA) kit (Monobind-AccuBind ELISA Kit- 96 wells)

-Bio-Gel P

-Mayer's Hematoxylin and Eosin Solution

-Gram's or Lugol's Iodine

-95% Alcohol

-Orange G Solution

-Polychrome Stain

Specimen collection and Handling

Blood samples were obtained by venipuncture and the serum was separated according to common procedures. Urine was collected as midstream urine in sterile urine container. Thereafter, it was concentrated passing through Bio-Gel P column and supernatant was collected. The samples were stored at -20°C for 24 hours. For longer period, samples were stored at -70°C or below. Samples were brought to room temperature before analysis.

Urine concentration method

Bio-Gel P gels are porous polyacrylamide beads prepared by copolymerization of acrylamide and N,N'-Methylene-bis-Acrylamide. The gels are extremely hydrophilic and essentially free of charge and provide efficient, gentle gel filtration of sensitive compounds. Their synthetic composition and freedom from soluble impurities preclude elute contamination. High resolution is assured by consistent narrow distribution of bead diameters and excellent molecular weight discrimination.

Dried gels having pore size of 90 to 180 μm were added to a measured volume of urine as weighed granules. Water and small molecules were attracted into the gel by osmosis. The exclusion limit of the Bio-Gel P is 6000 D, but those molecular weight > 6000 D were excluded by pore size.

It is almost always necessary to concentrate the urine before the test. The urine was taken and concentrated by passing it through Bio-Gel in 1 to 20 ratio. Fifty μg of Bio-Gel was taken in an aliquot and 1000 μL of urine was added and kept at 2° to 4°C for 5 hours. The supernatant urine will contain the CA19-9 molecules that are 210 kD and the low molecular weight particles will be absorbed by the gel. The supernatant of the urine sample were collected and tested for CA19-9 level by ELISA. Urine cytology was done by Papanicolaou Stain.

Procedure for measurement of CA19-9

The quantitative determination of CA19-9 concentration in human sample is done by a microplate immunoenzymometric assay. In this method, CA19-9 calibrator, patients' specimen or controls' are first added to a streptavidin coated well. Biotinylated monoclonal and enzyme-labeled antibodies (directed against distinct and different epitopes of CA19-9) are added and the reactants are mixed. Reaction between the various CA19-9 antibodies and native CA19-9 forms a sandwich complex that binds with the streptavidin coated well. After the completion of the required incubation period, the enzyme-CA19-9 antibody bound conjugate is separated from the unbound enzyme-CA19-9 conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known CA19-9 level permits the construction of a dose response curve of activity and concentration. By comparison to the dose response curve, an unknown specimen's activity can be correlated with CA19-9 concentration.

Statistical Analysis

Data were analyzed by SPSS software (the Statistical Package for the Social Sciences, version 14.0, SPSS Inc., Chicago, Illinois, USA) using student's independent sample *t* test and Mann-Whitney *U* test.

RESULTS

The mean age of the patients and controls was 58.74 ± 8.65 years and 61.76 ± 8.25 years, respectively. The mean urinary levels of CA19-9 and creatinine in patients were 194.59 ± 110.56 u/mL and 26.19 ± 9.32 mg/dL while they were 11.67 ± 8.42 u/mL and 63.05 ± 40.90 mg/dL in controls, respectively ($P = .0001$ and $P = .0001$) (Figure 1).

Of 47 patients, 35 had low-grade lesions (no invasion of the lamina propia), 10 high-grade lesions (extensive muscle involvement with distant metastasis), and 2 adenocarcinoma with deep muscle involvement.

Comparison of patients with low-grade and high-grade urothelial carcinoma is presented in

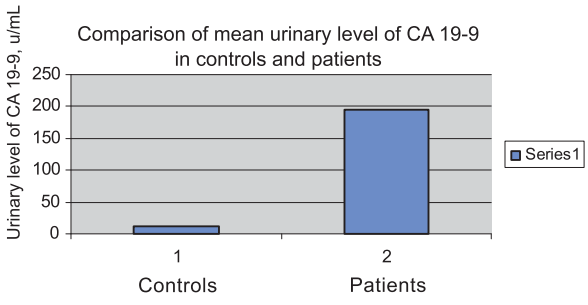

Figure 1. The bar diagram showing mean urinary level of CA19-9 between patients and controls.

Comparison between low-grade and high-grade transitional cell carcinoma

Category	Low-grade Urothelial Carcinoma	High-grade Urothelial Carcinoma
Mean urinary level of CA19-9, u/mL	206.56 ± 114.56	174.80 ± 94.06
Median urinary level of CA19-9 ,u/mL	188	188.15
Mean serum level of CA19-9, u/mL	25.86	12.99
Urinary cytology positive subjects	7	4

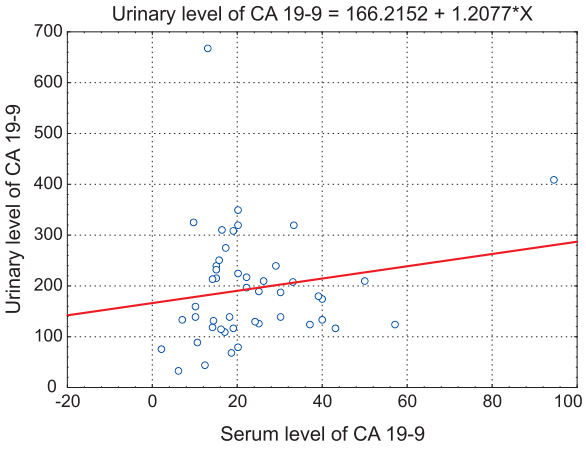

Figure 2. Scatter plot shows linear correlation between serum and urinary levels of CA-19.9 in patients.

Table. As it is shown, the mean urinary level of CA19-9 in patients with low-grade and high-grade bladder cancer was 206.56 ± 114.56 u/mL and 174.80 ± 94.06 u/mL, respectively ($P = .56$).

In Figure 2, Pearson's correlation showed poor linear correlation between serum and urinary level of CA19-9 in patients with the bladder cancer. Sensitivity and specificity of urinary CA19-9 were 71% and 82%, respectively.

DISCUSSION

The current standard diagnostic procedure for detection and follow-up of urothelial carcinoma of the bladder consists of urethrocystoscopy, which is the gold standard, and analysis of urine cytology in particular interval of time. Urethrocystoscopy is an invasive procedure and has a high risk of urinary tract infection.[13] Urine cytology is less sensitive in low-grade cases. Under this circumstance, the biomarker may play a role.[14,15]

It has been found that serum level of CA19-9 may be an important tumor marker in pancreatic cancer, but in early stage its value is questionable;[16] since a significant tumor size is required before the serum level is sufficiently high. Sakamato and colleagues demonstrated that the serum level of CA19-9 was not significantly raised in benign gut disorder while high incidence of raised concentration was observed in malignant gut lesion. Another study proposed that serum level of CA19-9 was extremely high in case of benign biliary tract disease.[17] In some papers, it has been shown that the urinary CA19-9 concentration is increased in case of normal epithelia of the bladder. [18]

Vriesema and associates determined that cystoscopy was 89% preferable over biomarkers when the sensitivity of urinary cytology was 90% or less.[19] In other study, it was found that urinary biomarkers have low sensitivity; however, their diagnostic accuracy was 90%. The researchers have reported the significant variation in the performance and characteristics of available biomarkers.[20,21] Theoretically, an ideal tumor marker should be highly sensitive, specific, and 100% accurate in differentiating between neoplastic and non-neoplastic lesions, and should be able to predict early recurrence.[22]

The significant finding was that the urinary level of CA19-9 was higher than the reference value in both high and low-grade lesions (Figure 2), but the urinary cytology was negative. The serum level of CA19-9 was also within the normal limit. The urinary level of CA19-9 was statistically significant ($P < .005$) and much higher in low-grade than in high-grade cases. But this study did not correlate the level of CA19-9 with the tumor progression. We did not compare the urinary biomarker with cystoscopy and other variety of biomarkers.

Maerstranzi and coworkers demonstrated that the urinary tract infection, especially associated with the urinary tract obstruction, significantly correlated with an elevated serum level of CA19-9 due to decreased clearance. But as this tumor

marker is produced by epithelial cell of the renal pelvis, the urinary level may or may not be acted in case of bladder cancer and should be needed for long-term study.[23] Patients with the bladder cancer mostly present with painless hematuria. In this study, we added hemoglobin as a cross-reactant to control sample, but no cross reaction was found in this assay.

Although cystoscopy is the main diagnostic tool in urothelial carcinoma, it is an invasive procedure and painful, and small peripheral tumors may be missed by an expert urologist.[24,25] Therefore, the long-term study is needed for urinary biomarkers as an important non-invasive diagnostic tool to overcome this problem. Studies are also needed for other tumor markers because the malignant process is known to elaborate a group of markers.

CONCLUSION

It can be concluded that urinary level of CA19-9 may play a diagnostic role in early stages of urothelial carcinoma of the bladder along with urinary cytology and cystoscopy. However, more extensive studies with greater number of patients are needed.

ACKNOWLEDGEMENTS

We are grateful to the Director, Ethics Committee, and Head of Department for giving permission to perform the study in this institution. We would also like to thank all the staff as well as patients of the Department of Urology for their cooperation.

CONFLICT OF INTEREST

None declared.

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