Role of NMP22 and major trace element in diagnosis of bladder cancer

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Abstract

Bladder cancer is the second most common cancer in the United States among genitourinary cancers. NMP-22 is a 240-kDa protein that is transitional cells specific that shed into the urine and has a 25-times higher concentration in the urine of bladder cancer patients than in non-cancer controls. Nowadays, trace elements are used as tumor biomarkers in different types of cancers. This study aimed to assay NMP22 (Nuclear Matrix Protein) and trace elements in bladder cancer patients. This case-control study was conducted at the Biochemistry Department / University of Baghdad/college of medicine. All patients were recruited from Gazi Al-Harery for Specialized Surgery during the period from October 2019 to February 2020. A total of 90 subjects were enrolled in this study and divided into two groups. The first group included 60 patients (52 male, 8 female) aged between 30-86 years and 34 of 60 patients who smoked for more than 1 year were defined as smokers. The highest number of patients with bladder cancer (35 patients) was hematuria (Present) while (25 patients) were non-hematuria. The second group included 30 healthy that have normal bladder tissue and without any previous history of any systemic diseases (non-smoker). NMP22 (Nuclear Matrix Protein), Trace element (zinc, copper, lead, iron, cadmium, manganese) were measured for diagnosis of bladder cancer. Results showed that the mean of serum NMP22 level in patients group (mean ± SD: 1210.2167 ± 669.88494) was significantly higher when compared to the mean of control group (mean ± SD: 383.7667 ± 107.47949) (P_value = 0.0001). Significant increase in serum (Cu, Cd, and Pb) levels (P_value = 0.0001) and significant decrease in serum (Zn, Fe, and Mn) levels (P_value = 0.0001) in bladder cancer patients when compared with normal group. The mean of serum iron level in patients (hematuria) group (mean ± SD: 56.3143 ± 6.43415) was significantly lower when compared to the mean of patients (non-hematuria) group (mean ± SD: 96.6800 ± 13.59755) (P_value = 0.0001). Conclusion: the mean of serum NMP22 level in patients group of bladder cancer was significantly higher when compared to the mean of control (healthy) group. It is easily available and economical, and it causes no patient discomfort. Low serum (Zn, Fe, and Mn) and high (Cu, Cd, and Pb) levels in bladder cancer patients when compared with control. The mean of serum iron level in patients (hematuria) group was significantly lower when compared to the mean of patients (non hematuria) group.

Keywords Bladder cancer, NMP22, Trace elements

Introduction

Bladder cancer is the second most common cancer in the United States among genitourinary cancers. In the world, it is the 11th most common cancer among all cancers. The worldwide standardized incidence for age was 8.9 for males (100,000 person-year) and 2.2 for females (2008 data), while it was 27 for males and six for females in Europe (Yıldızhan & Gemalmaz, 2019). Diagnosis of bladder cancer is made by cystoscopy, cytology and pathological evaluation of the excised material. If bladder cancer was detected or urinary cytology was found positive, diagnostic cystoscopy is omitted and biopsies from suspected areas or tumor resection are performed under anesthesia (Bahjuk, Böhle, Burger, et al., 2014). The “gold standard” diagnostic procedure for BCa is cystoscopy, a technique that can be invasive, expensive, and a possible cause of urinary tract infection (Gogalic et al., 2015; Herr, 2015). In clinical practice, cystoscopy is accompanied by urine cytology. This plays a more important role in the case of high-grade tumors and carcinoma in situ (CIS). The main problem with cytology is its low sensitivity for low-grade tumors, although it does have an acceptable specificity (Yafi et al., 2015). Many molecular diagnostic tests have been developed to complement or replace cytology and cystoscopy. Assays that measure single protein markers such as BTA stat, BTA TRAK and NMP22 Bladder Check often show low sensitivity and/or specificity (Ellen et al., 2018). Several noninvasive biomarkers such as nuclear matrix protein 22 (NMP22) test, have been approved by the Food and Drug Administration (FDA), albeit with performance rates remaining insufficient to replace or to guide current diagnostic methods (Chengtao et al., 2018).

Nuclear Matrix Protein 22 (NMP22) is a non-histone chromatic protein that belongs to the bladder cancer-specific nuclear mitotic proteins. NMP22 is responsible for the proper position of chromatids during mitosis and final separation of daughter cells. It has an important role in ribonucleic acid (RNA) synthesis, deoxyribonucleic acid (DNA) transcription and replication, and in morphological changes of nuclear structure; therefore, it can also have a huge influence on the genes that are crucial in carcinogenesis (Beata et al., 2017). NMP-22 is a 240-kDa protein that is transitional cells specific that shed into the urine and has a 25-times higher concentration in the urine of bladder cancer patients than in non-cancer controls (van Rhijn et al., 2005). Trace elements are the main components in biological structures. They play an important role in the modulation of OS and DDR/repair mechanisms (Lee, 2018; Sharif et al., 2012). However, some trace elements can also be toxic when exceeding body requirements (Kaba et al., 2014). Recently, studies have indicated that some trace elements have an essential role in the process of malignant tumor incidence and progression (Shang et al., 2014; Kohzadi et al., 2017). Nowadays, trace elements are used as tumor biomarkers in different types of cancers (Lee et al., 2009). Our body cells are perpetually exposed to thousands of DNA lesions, which may be due to endogenous or exogenous agents, and these stressors lead to decreased genomic stability (Tehrani et al., 2018). This study aimed to assay NMP22 (Nuclear Matrix Protein) and trace elements in bladder cancer patients.

Material and Methods

This case-control study was conducted at the Biochemistry Department / University of Baghdad/college of medicine. All patients were recruited from Gazi Al-Harery for Specialized Surgery during the period from October 2019 to February 2020. A total of 90 subjects were enrolled in this study and divided into two groups. The first group included 60 patients (52 male, 8 female) aged between 30-86 years and 34 of 60 patients who
smoked for more than 1 year were defined as smokers. The highest number of patients with bladder cancer (35 patients) was hematuria (Present) while (25 patients) were non-hematuria. All patients were first diagnosed with bladder tumor and investigated by an urologist and underwent either cystoscopy examination for transurethral resection of bladder tumor (TURB) or cystoscopy with biopsy of bladder lesion for histopathological examination. The second group included 30 healthy that have normal bladder tissue and without any previous history of any systemic diseases (non-smoker).

About 10 ml of venous blood was collected between 9:00 A.M to 1:00 P.M from each subject. About 2.5 ml of blood was dispensed into plastic tubes containing EDTA to estimate lead and cadmium. The remaining blood part was dispensed into plane tube, allowed to clot and then centrifuged at 3000 rpm for 10 minutes to obtain sera which was transferred into another labeled tubes and stored frozen at -80°C until estimation for Nuclear Matrix Protein (NMP22) and trace elements were performed.

Serum NMP22 was measured using Human Nuclear matrix protein 22(NMP22) ELISA Kit. This kit uses enzyme-linked immune sorbent assay (ELISA) based on biotin double antibody sandwich technology to assay Human Nuclear matrix protein 22(NMP22).

Assessment of inorganic elements (Zn, Cu, Pb) was performed by Flam atomic absorption spectrometry (FAAS) while (Cd, Mn) was performed by graphite furnace atomic absorption spectrometry (GFAAS). Serum Iron level were determined using enzymatic colorimetric methods (spectrophotometry).

Statistical analysis

Statistical analysis was performed with the IBM SPSS 23 statistical software program. Univariate data were summarized using standard descriptive statistics, tabulation of categorical variables and histograms of numerical variables. Mann Whitney Test (a non-parametric equivalent of the independent samples t-test) was used to compare means of continuous variables. Exact tests were used to calculate the p value. In all statistical analyses, a p value < 0.05 was considered significant.

RESULTS AND DISCUSSION

The mean of serum NMP22 level in patients group (mean ± SD: 1210.2167 ± 669.88494) was significantly higher when compared to the mean of control group (mean ± SD: 383.7667 ± 107.47949) (P value= 0.0001) (Fig 1). These results were agreed with Beata Szymańska et al, who reported a statistically higher level of NMP22, expressed as ng/mg creatinine, in patients with bladder cancer than in the control group (p = 0.005) (Beata et al., 2017). Many studies have reported the diagnostic value of the NMP22 test in detecting bladder cancer, but these studies have shown mixed results. In this meta-analysis, we combined 24 separate studies consisting of 8848 patients to evaluate the diagnostic value of the NMP22 test in detecting bladder cancer. The results showed that NMP22 test might be appropriate for detecting bladder cancer (Lei et al., 2012).
The mean of serum zinc level in patients group (mean ± SD: 66.4833 ± 7.92227) was significantly lower when compared to the mean of control group (mean ± SD: 111.2333 ± 15.40193) (P-value = 0.0001) (Fig 2). These results were agreed with İlhan Gecit et al, who reported a Mn and Zn levels were found 0.0750± 0.049 and 0.956± 0.39 in group control to 0.0475± 0.016, and 0.6157± 0.3263 0.24 in group bladder cancer, respectively. The amounts of these elements were decrease between two groups (P< 0.005) (İlhan et al., 2011).
The mean of serum copper level in patients group (mean ± SD:163.983± 14.40161) was significantly higher when compared to the mean of control group (mean ± SD:93.300 ± 8.48183) (P value= 0.0001) (Fig 3). These results were agreed with Sura Dhafir Dawood et al, who reported a highly significantly serum levels of Cu was demonstrated in our results for bladder cancer patients (mean ±SD: 31.76 ± 1.90 μmol/L ) respectively when compared with control groups (mean ± SD: 19.17 ± 1.78 μmol/L ) (P value < 0.0001) (Sura et al., 2010).

Figure (3) Comparison of copper level between patients and controls

The mean of serum cadmium level in patients group (mean ± SD:0.42733 ± 0.079997) was significantly higher when compared to the mean of control group (mean ± SD:0.16233 ± 0.027378) (P value= 0.0001) (Fig 4). These results were agreed with İlhan Gecit et al, who reported The Cd, Ni and Co element levels were significantly higher in bladder cancer group (P < 0.005) (İlhan et al., 2011).

Figure (4) Comparison of cadmium level between patients and controls

The mean of serum lead level in patients group (mean ± SD:25.7333 ± 4.86728) was significantly higher when compared to the mean of control group (mean ± SD:13.2667 ± 2.31834) (P value= 0.0001) (Fig 5). These
results were agreed with Sven Wach et al., who reported studied a validation cohort that consisted of 21 BCa patients, 29 non-tumor bladder patients and 18 healthy controls. We detected 15 elements with significantly higher concentrations (boron, calcium, cadmium, copper, chromium, potassium, lithium, magnesium, nickel, lead, sulfur, strontium, titan, vanadium, and zinc) and two elements with significantly lower concentration (iron, molybdenum) when comparing BCa patients and healthy controls (Sven Wach et al., 2018).

The mean of serum iron level in patients group (mean ± SD:73.1333 ± 22.40117) was significantly lower when compared to the mean of control group (mean ± SD:138.2000 ± 32.44029) (P value = 0.0001) (Fig 6). These results were agreed with Sura Dhafir Dawood et al, who reported a significantly reduction in serum Se, Fe, and Zn levels of bladder cancer patients (mean ± SD: 0.34 ± 0.05; 1.45 ± 1.01; 3.59 ± 35) μmol/L, when compared with control groups (mean ± SD: 1.03 ± 0.15; 17.27 ± 1.25; 15.51 ± 2.21) μmol/L, (P value: < 0.0001) (Sura et al., 2010). Two elements with significantly lower concentration (iron, molybdenum) when comparing BCa patients and healthy controls (Sven Wach et al., 2018).
The mean of serum manganese level in patients group (mean ± SD:0.08533 ± 0.019351) was significantly lower when compared to the mean of control group (mean ± SD:0.21400 ± 0.041323) \( (P_{\text{value}}= 0.0001) \) (Fig 7). These results were agreed with İlhan Gecit et al, who reported Mn and Zn levels were found 0.0750± 0.049 and 0.956± 0.39 in group control to 0.0475± 0.016, and 0.6157± 0.3263 0.24 in group bladder cancer, respectively. The amounts of these elements were decrease between two groups \( (P< 0.005) \) (İlhan et al., 2011).

**Figure (7): Comparison of manganese between patients and controls**

The mean of serum iron level in patients (hematuria) group (mean ± SD:56.3143 ± 6.43415) was significantly lower when compared to the mean of patients (non-hematuria) group (mean ± SD:96.6800 ± 13.59755) \( (P_{\text{value}}= 0.0001) \) (Fig 8). These results were agreed with İlhan Gecit et al, who reported that serum iron levels in patients with invasive bladder cancer were found to be significantly lower when compared with non-invasive group. Anemia as a result of chronic blood loss and chronic hematuria is one of the mechanisms that may cause a lower iron level in the bladder cancer group (İlhan et al., 2011).

**Figure (8): Comparison of iron level between BC patients(hematuria and non- hematuria**
Conclusion

Serum NMP22 level in patients group of bladder cancer was significantly higher when compared to the mean of control (healthy) group. The NMP22 test is an in vitro immunoassay used for the quantitative detection of NMP22 in serum. It is easily available and economical, and it causes no patient discomfort. We recommend to more extensive study of NMP22 in superficial tumors of urinary bladder and to use it as an adjunctive method beside urinary cytology and cystoscopy for surveillance of patients with superficial bladder cancer.

Trace elements (Cu, Pb and Cd) showed a highly significant while trace elements (Zn, Fe and Mn) showed a lower significant in the serum of patients group of bladder cancer when compared to control(healthy) group. The trace elements should be monitored routinely in bladder cancer patients who could be helpful in improving the general health conditions and reducing progression of the disease.

The mean of serum iron level in patients (hematuria) group was significantly lower when compared to the mean of patients (non-hematuria) group.

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