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Evaluation of Serum Bladder Cancer Specific Nuclear Matrix Proteins - 1 levels in Non-Muscle Invasive Bladder Cancer Patients

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ABSTRACT

Background: The most common type of bladder tumor found in its early stages is non-muscle invasive bladder cancer. Diagnosis of bladder carcinoma is made using cytology and cystoscopy, and it is difficult for clinicians to make due to a lack of sensitivity and specificity. Biomarkers hold the promise of improving diagnostic and screening methods. Bladder cancer specific nuclear matrix protein-1 (BLCA-1) has recently been identified as a marker for bladder cancer, because it is abnormally released in the blood of bladder cancer patients. We assess the usefulness of serum BLCA-1 levels as tumor markers in the diagnosis of non-muscle invasive bladder cancer. **Method:** At Damietta Cancer Institute in Damietta, Egypt, 50 patients and 30 healthy subjects were recruited and pathologically diagnosed. Subjects are classified into three groups: Ta non-muscle invasive bladder cancer group (Ta NMIBC) patients (n = 26), T1 non-muscle invasive bladder cancer group (T1 NMIBC) patients (n = 24) and healthy subjects group (n= 30). Serum samples were collected, and BLCA-1 levels measured using an enzyme-linked immunosorbent assay, and the statistical analyses were used to uncover the relationships. **Results:** Significant increase in BLCA-1 levels in serum of NMIBC patients with more T1 levels elevation than Ta NMIBC, $p < 0.001$. **Conclusions:** We conclude that serum BLCA-1 detection has value in the diagnosis of NMIBC, with higher levels in T1 NMIBC than Ta NMIBC. As a result, BLCA-1 is expected to be a unique marker for detecting NMIBC patients.

1. Introduction

Bladder cancer is the world's second most common genitourinary tract cancer, with significant morbidity and mortality (Siegel R et al., 2018). As flat tumors, papillary tumors are well differentiated, whereas high grade tumors are poorly differentiated and highly aggressive. The diagnosis of bladder cancer has not changed dramatically in the last few decades. Maclennan S et al state that cystoscopy is still the gold standard modality for diagnosing and monitoring bladder cancer(Maclennan S et al., 2011). It is, however, a very invasive procedure. As a result of the high recurrence rate and frequent need for follow-up, this could imply a significant financial burden on individuals and families. Cytological examinations are another additive approach to diagnosis; however, their main limitation is poor sensitivity, particularly for low-grade tumors, with various studies reporting values ranging from 11% to 76% (Kevin K et al., 2017).

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Urinary cytology has a diagnostic sensitivity of only 16% for low-grade NMIBC (Faysal A et al., 2015).The primary goal of biomarker discovery is to create cost-effective, non-invasive strategies for the detection of bladder tumors. Urinary biomarkers for bladder cancer detection have long been sought by researchers as a potential alternative to cystoscopy and cytology (Rosser C.J. et al., 2013).

All cancer cells share the property of aberrant nuclear shapes and abnormal nucleoli. Because these alterations are so common in cancer cells, they are frequently used as a pathological marker of transformation. The nuclear matrix, at least in part, determines nuclear shape, which reflects the internal nuclear structure and processes (Pienta K. J. et al., 1989). Nuclear matrix is crucial in the regulation of critical cellular processes like DNA replication and transcription (Getzenberg R. H. et al., 1994). The nuclear matrix is the scaffolding or framework of the nucleus, consisting of peripheral lamins and pore complexes, an internal ribonucleic protein network, and residual nucleoli (Berezney R et al., 1974). The nuclear

matrix contains 10% of the nuclear proteins but no lipids, DNA, or histones (Fey E. G. et al., 1991).

Although the majority of known nuclear matrix proteins are shared by all cell types and physiological states (Kantor A. F. et al., 1984), some nuclear matrix proteins appear to be unique to specific cell types or states.

The nuclear matrix is the cell nucleus' three-dimensional support structure. It serves a number of critical roles in a wide range of cellular processes (Replogle-Schwab et al., 1996). They involve determining the shape of the nucleus, organising DNA throughout cell cycle, stabilising and orienting DNA during replication, arranging gene regulatory complexes, and synthesising RNA.

The nuclear matrix's protein structure is tissue-specific and can act as a "fingerprint" for each kind of cell and/or tissue (Getzenberg R. H., Coffey D. S., 1990). Mitogenic stimulation and differentiation induce changes in the composition and structure of nuclear matrix proteins (Dworetzky S et al., 1990).

Six nuclear matrix proteins (BLCA-1 through 6) that are unique to patients with transitional cell carcinoma and three nuclear matrix proteins that are specific to normal bladder tissue but absent in cancer patients were identified in an effort to develop a specific and sensitive method for detecting bladder cancer. These proteins were not discovered in any of the other cancerous or normal tissue types investigated. Based on their abundance, three of the six proteins identified were chosen for sequencing (Getzenberg et al., 1996).

Nuclear matrix proteins are specifically displayed in bladder cancer tissues and can be released into the blood when cancer cells are lysed are recognized as bladder cancer-specific nuclear matrix proteins (BLCAs), and they have been associated with tumor cell proliferation and angiogenesis (Konety B.R. et al., 2000). According to earlier studies (Getzenberg R.H. et al., 1996) and (Myers-Irvin J.M. et al., 2005), the BLCA family of proteins has a high level of sensitivity and specificity in the diagnosis of bladder cancer and has a great potential for use in clinical diagnostic applications.

Subjects and Methods

1- Subjects.

In this study, patients were first diagnosed pathologically at the Damietta Cancer Institute, Pathology Department, Damietta, Egypt.

Bladder cancer can divided into NMIBC and MIBC, non-muscle invasive bladder cancer (NMIBC), which is limited to the mucosa layer (Ta) or sub mucosa (T1), or CIS. MIBC as T2, T3 and T4 as shown in figure 1. (Babjuk M et al., 2022).

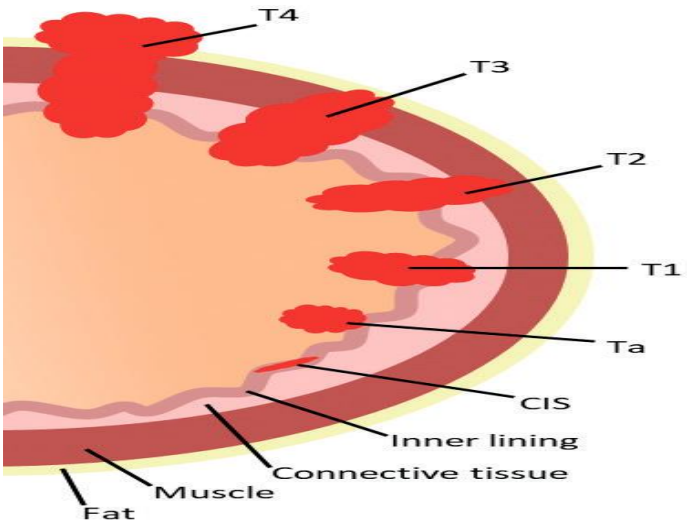

Figure 1: Bladder cancer staging. (Babjuk M et al., 2022).

Patients were recruited and divided into two groups: Ta NMIBC patients (papillary non-invasive tumor) (n = 26), and T1 NMIBC patients (tumor invades the inner lining and connective tissue) (n = 24), and thirty cancer-free, healthy participants were included. The 26 patients with Ta NMIBC include 22 males, 4 females, and the mean age was 58±8 years. The 24 patients with T1 NMIBC include 17 males and 7 females, and the mean age was 60±9 years. The 30 healthy subjects include 21 males and 9 females, and the mean age of 54±11 years. CIS NMIBC not included in this study due to the low sample size. Serum samples were collected from all subjects.

Clinical data of patients including age, sex, tumor pathology and stage were collected from medical records. TNM(Tumor, Nodes and Metastasis) classification system for bladder cancer was utilized to play out the pathological staging of the study (Amin M.B. et al., 2017).

The diagnosis of patients was done by the Damietta Oncology Center's pathologists as Ta NMIBC and T1 NMIBC . Figure 2

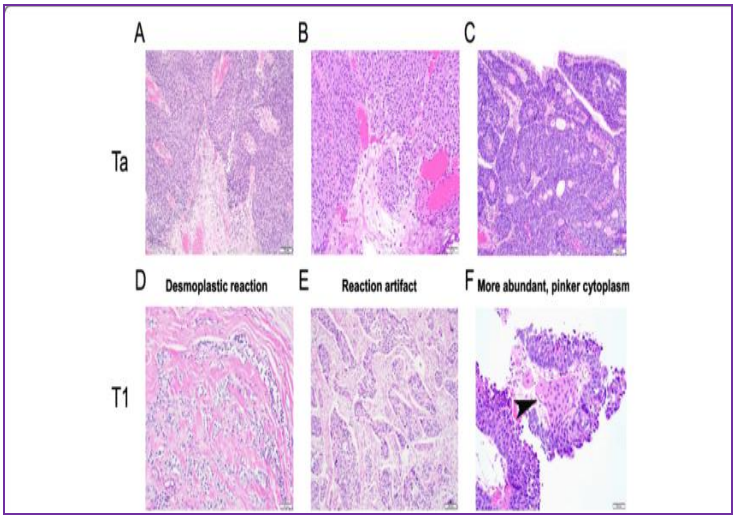

Figure 2: Histological characteristics of bladder cancer stages Ta (a-c) versus T1 (d-f).

All samples had their serum BLCA-1 levels measured using an enzyme-linked immunosorbent assay (ELISA). Statistical analyses were used to reveal the associations therein.

2- Samples Preparation:

Venous blood samples (5–10 ml) were collected, and then centrifuged at 3500 rpm for 20 min, serum were stored at −80°C.

3- Enzyme-linked immunosorbent assay

Serum BLCA-1 levels are determined by enzyme-linked immunosorbent assay (ELISA), (SHANGHAI KORAIN BIOTECH CO., LTD, SHANGHAI, CHINA), Cat. No E7905Hu.

Statistical analysis

Mean, SD, and T-test are used to explain the continuous variables with normally distributed data. The diagnostic power of the markers were examined by ROC analysis, and comparisons between groups were made using the ANOVA test. The statistical programme SPSS version 15.0 was used to examine the data (SPSS Inc., USA). P values under 0.05 were regarded as statistically significant alpha values (Levesque R., 2007).

2-Results

BLCA-1 levels in serum of Ta bladder cancer patients were significantly elevated (7.3±2.9 ng/ml, P<0.001), compared to the control group (4.47±2.1 ng/ml). Furthermore, our findings revealed a significant increase in BLCA-1 levels in the serum samples of T1 NMIBC patients (9.2±4.0 ng/ml, p < 0.001) compared to the control group (4.47±2.1 ng/ml). Figure 3, Table1

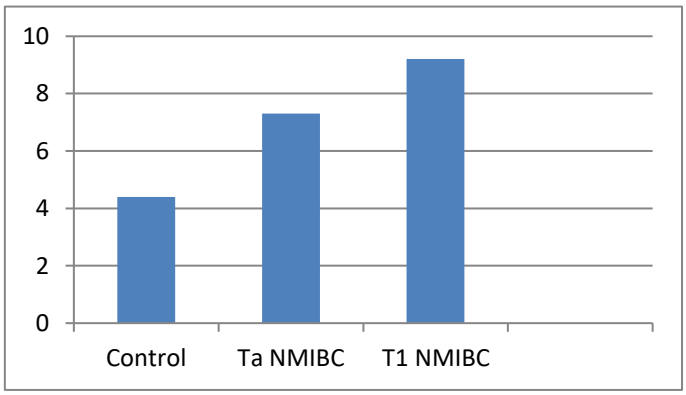

Figure 3: Serum BLCA-1 concentration in control group, Ta NMIBC group and T1 NMIBC patients group.

Table 1: Serum BLCA-1 concentration in control group, Ta NMIBC group and T1 NMIBC patients group.

Marker	Control	Ta	T1
BLCA-1 (Serum) P value	4.47 ±2.1	7.3±2.9 P<0.001	9.2±4.0 P<0.001

The P value was not significant in comparison between serum BLCA-1 levels in Ta, T1 NMIBC since, P value was 0.64. (Table 2)

Table 2: Comparison between serum BLCA-1 in Ta NMIBC and T1 NMIBC patients and their P value.

Marker	Ta	T1	P value
BLCA-1 (Serum)	7.3±2.9	9.2±4.0	P =0.64

ROC analysis revealed that the AUC in Ta NMIBC patients' serum was 0.67 (95% CI: 0.52-0.8) P<0.001, with sensitivity of 61.5%, and specificity of 70%, respectively. Table 3, Fig 4

Table 3: ROC curve evaluation between serum BLCA-1 in control group and that of Ta NMIBC patients group.

Marker	AUC	Std. Error	95% CI	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
BLCA-1 Serum	**0.67**	**0.079**	**0.52 - 0.82**	**61.5**	**70**	**64**	**67.7**	**66**	**P<0.001**

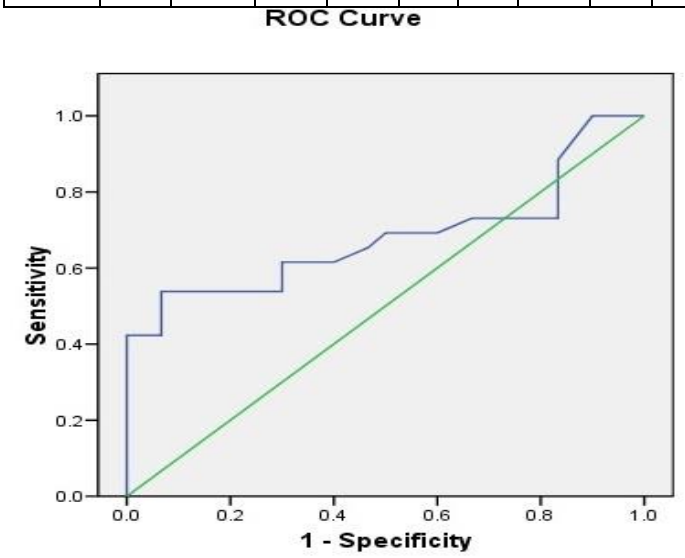

Figure 4: ROC curve for serum BLCA-1 in control group Versus Ta NMIBC patients group.

ROC analysis revealed that the AUC in T1 NMIBC patients' serum was 0.84 (95% CI: 0.72-0.95) P< 0.001, with sensitivity of 79.4%, and specificity of 73%. Table 4, Fig 5.

Table (4): ROC curve evaluation between serum BLCA-1 in control group and that of T1 NMIBC patients group.

Marker	AUC	Std. Error	95% CI	Sensitivity	Specificity	PPV	NPV	Accuracy	P value
BLCA-1 Serum	**0.84**	**0.060**	**0.72 - 0.95**	**79.4**	**73**	**67.8**	**80.7**	**74**	**P< 0.001**

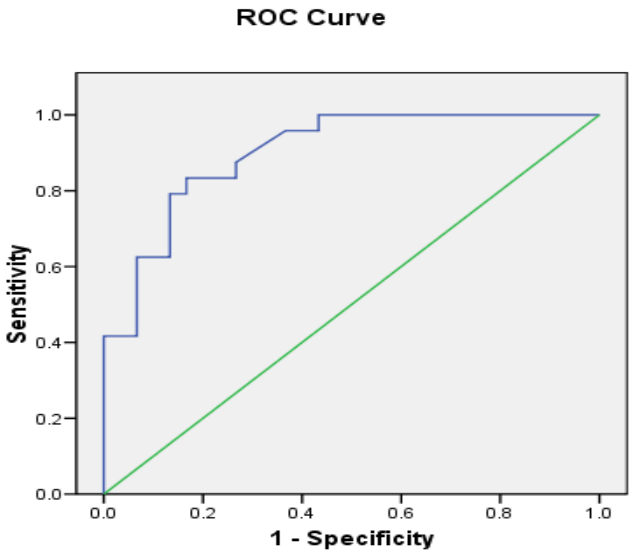

Figure 5: ROC curve for serum BLCA-1 in control group Versus T1 NMIBC patients group.

Discussion

In our study, we noticed that BLCA-1 levels in serum of NMIBC patients were significantly higher than those of healthy controls who were free of malignancy, and marker expression was also correlated with disease severity, with T1 being higher than Ta. Our results strongly suggest that BLCA-1 is involved in the upsurge and subsequent development of NMIBC.

In addition, another study used Western blot and ELISA to look at BLCA-1 expression in urine and bladder tissue. They discovered that BLCA-1 protein levels were significantly higher in bladder cancer patients than in healthy subjects, but that this did not correlate with tumor grade. They achieved 80% sensitivity and 87% specificity, demonstrating the utility of a BLCA-1 in the diagnosis and monitoring of bladder cancer patients (Myers-Irvin J.M. et al., 2005). This may open the door to the identification of biomarkers and the use of body fluids for tumor diagnosis and prognosis.

Theoretically, BLCA-1, a nuclear matrix protein, could be released into the bloodstream after the lysis of tumor cells. There are few published reports on using the serum BLCA-1 test to diagnose bladder cancer, and the vast majority of BLCA-1 studies have utilized urine samples (Zhiyong Wang et al., 2018). In order to determine whether BLCA-1 could be detected in the serum of NMIBC patients and whether its high expression in serum indicated a high risk of bladder cancer, we carried out this study. Our findings indicate that serum BLCA-1 levels are linked to NMIBC with a sensitivity of 61.5% and 79.4% and a specificity of 70% and 73% for Ta NMIBC and T1 NMIBC, respectively. This implies that BLCA-1 may be significantly involved in tumorigenesis.

Shifts in nuclear structure can influence gene expression, and thus play an important role in carcinogenesis (Konety B.R et al., 1998). Because BLCA-1 is a nuclear matrix protein specific to bladder cancer, it is theoretically involved in tumorigenesis and may increase with tumor stage, as shown in our work, the mechanism of action may entail the expression of various inflammatory cytokines and mediators.

BLCA-1 expression was linked to the expression of a number of inflammatory cytokines and mediators, including MMP9, VEGF, IL-8 and IL-1, but not with the secretion of TNF, according to a study that looked into the potential relationship between BLCA-1 levels and clinically significant pathological parameters of bladder cancer (Feng C et al., 2015). Furthermore, it is known that the nuclear matrix is inextricably linked to the cytoskeleton and extracellular matrix (ECM), and that its extracellular framework can functionally alter a cell's genetic expression and response to various triggers such as hormones and growth factors (Getzenberg et al., 1991). The nuclear matrix interacts with the ECM as well, and its protein composition is regulated by these interactions at least in part.

When the extracellular matrix is altered, such as by adjacent malignant tumours, the nuclear matrix shows removals and additions of specific proteins (Getzenberg et al., 1991). These changes may incorrectly amplify or delete active genes in a cell, as well as potentially stimulate the expression of oncogenes or decrease tumor suppressor genes.

The nuclear matrix also regulates gene expression via steroid hormone receptor binding sites. Numerous hormone-responsive tissues have been shown to have distinctive receptors within their nuclear matrices that promote hormonally regulated gene activation by connecting to hormone response elements in DNA (HREs). In turn, HREs are reported in the promoter regions of hormonally regulated genes, where they control gene transcription. Furthermore, these matrix-associated receptors are not present in non-steroid responsive tissues and have been shown to be down-regulated during times of low hormonal activity (Getzenberg et al., 1990). Numerous studies have shown that NMPs can be phosphorylated, implying a link between gene expression regulation and the nuclear matrix through one of the most common cellular control mechanisms.

Our study found a link between nuclear matrix protein-1 levels in serum and the stage of the disease, which supports our belief that BLCA-1 levels can rise in the blood after tumor lysis and so could also in patient's urine.

The findings of this study need to be confirmed and validated. In order to improve statistical confidence in the study's results, a wider cohort of study participants is required to confirm the clinical utility of BLCA-1 as a marker in NMIBC patients. Furthermore, comparing marker levels in follow-up patients could have been beneficial.

Conclusions

We come to the conclusion that serum BLCA-1 detection, with greater levels in T1 NMIBC than Ta NMIBC, has value in the diagnosis of NMIBC. Therefore, it is anticipated that BLCA-1 detection will be a unique biomarker for identifying NMIBC patients.

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