

Prognostic value of CK14 expression in urinary bladder cancer

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Abstract:

The aim of current study is to determine the expression of the CK14 protein in human bladder cancer, and demonstration of correlations between this parameter and clinical pathologic variables as (grade and stage of tumor), and also by using this marker can classify bladder cancer in to basal and luminal type. The current study is designed to detect the role of Ck14 expression in bladder carcinoma as a possible marker for detecting the biological behavior of malignancy and its correlation with grade and muscle invasiveness for both diagnostic and prognostic purposes. The study focuses on a technique of immunohistochemistry for detection Ck14 expression in bladder cancer. The samples are collected randomly in southern Iraq, in AL-Nasiriya city, from AL Hussein teaching hospital. Number of samples is (100) samples, (70) bladder cancer tissue and (30) controls benign tissue. Results of this study reveal that Ck14 expression is positive in 45 out of 70 sample. The study present showed Ck14 expression is increased in high grade bladder cancer represent (86.0%), while in low grade (29.6%), CK14expression high in advanced tumour stages T3, T4 percentage (100%) and T2 stage (87.2%), expression of CK14 low in early tumor stages Ta (26.3) T1(33.3). expression CK14 excessive in muscle invasive (97.6%), whereas the expression CK14 is low in non-muscle invasive type (28.6%). So Ck14 can be used as a marker for assessment of bladder cancer aggressiveness. This study represents an important step because there is absence of studies about this topic in Iraq.

Key words: Bladder cancer, CK14, Immunohistochemical.

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INTRODUCTION

One of the most prevalent cancers of the urinary tract, Bladder Cancer (BCa) is expected to cause 17,980 deaths and 80,000 new diagnoses globally in 2020. bladder cancer has mutation histological variants, The conventional urothelial

carcinoma also called as transitional cell carcinoma accounts for the majority of invasive bladder malignancies, [1,2]. Bladder cancer is a disease in which the cells lining the urinary bladder lose the ability to regulate their growth and start dividing uncontrollably This abnormal growth results in a mass of cells that form a tumor [3]. Bladder Cancer also referred to as urological cancer has been defined increasing occurrence particularly in advanced countries [4]. Bladder cancer is the most common pathological conditions among tumors of the urinary system that require a combination of several techniques and immunological tests to reach the type of tumor, and despite the development of pioneering techniques in the field of medicine, the exact diagnosis of the case has not been made without relying on taking a biopsy from bladder tissue [5]. Types of bladder cancer included Transitional Cell Carcinoma (TCC) is the most common type about (97%), followed by Squamous Cell Carcinoma (SCC) about (2%) and the lowest frequency is adenocarcinoma as (1%) [6]. Bladder cancer is the fourth most common cancer in men, with approximately 60,000 new diagnoses each year ranking as the eighth leading cause of cancer related deaths in the United States, with about 12,000 deaths annually, Specifically, in 2017, there were 79, 030 cases of bladder cancer and 16,870 associated deaths in the United States [7]. In Iraq, bladder cancer is the fifth of ten cancers, the third cancer in men and the eighth in women. 866 men were diagnosed with bladder cancer, compared to 297 women, according to the Iraqi Cancer Register [8]. As for the year 2021, it will be ranked fifth among other most common cancers, with an infection rate of 1,769, as the number of male infections is 1,360, while females are 409, and this indicates that male infections are higher than females [9]. Generally, urothelial carcinoma is categorized into Non-Muscle-Invasive Bladder Cancer (NMIBC) and Muscle-Invasive Bladder Cancer (MIBC) according to bladder wall invasion [10]. While (NMIBC) generally has a low risk of distant metastasis, (MIBC) is more aggressive and is more likely to metastasize. (MIBC) usually requires intensive management, which includes radical cystectomy with perioperative chemotherapy [11,12]. Despite of complete surgery and adjuvant therapy, the 5-year overall survival of MIBC is approximately 36% [11]. Diagnosing bladder cancer is more difficult as patients may not exhibit severe symptoms Typically it only manifests as painless hematuria which is not specific bladder malignancy [13-15]. Urinary biomarkers offer method to screen for bladder cancer, Cytokeratin (CK14) has been extensively studied in cases of malignancies.

These proteins form a class of intermediate filaments which contribute in the forming of epithelial cells. Previously cytokeratin's (CKs) are believed to represent the occurrence of apoptosis and cell proliferation, or necrosis [16]. Among the 20 types of CKs, CK 14 is a marker of squamous epithelial cells differentiation. Many studies have analyzed CK 14 as diagnostic or prognostic marker in various cancers. CK14 expression can be detected by immuno assay method [17,18]. Another study referred the most suitable criterion for histochemical for the identify of basal like breast carcinoma by basal markers was evaluated for ck14 expression invasive breast carcinoma to determine their sensitivity and specificity for basal breast cancer, CK14 proved is a reliable option to distinguish the basal phenotype [19].

MATERIALS AND METHODS

Collection of Samples

Samples include (100) of bladder tissue formalin fixed paraffin, it divided into two groups (70) samples (patients' group) of bladder cancer tissues and (30) samples (control group) of benign bladder lesion. The age ranges is between (20-80) years old. All samples were collected from educational Al Hussein hospital and a private laboratory in Thi-Qar province during the period between July 2022 and February 2023. It samples were fixed in 10% formalin when starting of collection for histopathological and immunohistochemical analysis.

Immunohistochemistry assays (IHC)

In this study the immunohistochemical technique was been performed as described earlier [20]. First step is the deparaffination, accomplished by soaking samples in xylene and then rehydrated through a series of graded ethanol alcohol (70%, 90%, 100 %) After that, Antigen retrieval by placing the slides in Retrieval Solution in high temperature. Then left in room temperature for 20 min to cooled, subsequently addition of peroxidase to remove internal peroxidase activity of tissue for 10 min and slides were again washed 3 times for 10 min in phosphate buffered saline. Then let sections overnight for incubated with primary antibodies Cytokeratin 14 (CK14 - mouse monoclonal antibody) (CK14; 1:50). In the next day, the slides subjected to washing 3 times with (PBS) for 10 min. then dropping of anti-mouse labeled polymer-HRP primary antibody and incubated then at room temperature with gentle vibrator for 30 min. Then, the slides washed three times with PBS buffer for 5 min each. After that drops DAP Chromogen additional to the slides and incubated for 10 min at room temperature according protocol Dako. Next step, slides washed with D.W (distilled water) for 10 min. Then, drops of the counter stain haematoxylin was applied onto the slides and let for incubation at room temperature for 2 min, to stain the nucleus of cells, then, slides washed

or rinsed by the running tap water for 2 min and distilled water for 1 min. After that, the slides dehydrated by soaking in ascending concentrations of alcohol (70%, 90%, 100%) for 1 min. each one. After dehydration slides soaked in xylene 2 times for 2 min each. Then drops of DPX applied onto slides and covered with cover slides. CK14 expression was scored with the Allred scoring system, cytoplasmic expression intensity was graded 0 to 3 least to most intense and area with positive expression was score from 0 to 5 (0 = none, 1=1%, 2= 10%, 3=33%, 4=66, 5=100% cells positive)

Selection of markers for basal subtype

A single marker has been selected to represent the basal subtypes We selected CK14 as the basal marker because it is a marker known for urothelium and stem cells [21]. and CK14 is overexpressed in the bladder cancers of the basal subtype [22].

Statistical analysis

The data has been analyzed by the statistical package for available from SPSS percentages are used to display the data. The Pearson Chi-square test is used to determine the significance of the differences between significant qualities (qualitative data). Whenever the P-value is less than 0.05, statistical significance is considered.

RESULTS

The results of the current study showed that (45) case CK14 positive expression out of (70) bladder cancer show over expression of CK14 and 2 out of 30 controls are positive for CK14. In (Table 1) High expression of CK14 in advanced tumor stages T3, T4 percentage (100%) and (87.2%) T2 stage, and decreased in the early tumor stages Ta (26.3%), T1 (33.3%) (Figure 1). The results explained (8) cases of (45) positive CK14 expression of bladder cancer is of low-grade urothelial carcinoma and high expression CK14 in high grade of bladder cancer (37) cases as in (Table 2). This study shows over expression of CK14 in muscle invasive of bladder cancer (41) case out of 45 while low expression of CK14 in non-muscle invasive bladder cancer (8) case as in. (Table 3). The finding of the study shows the positive CK14 expression is for basal subtype of bladder cancer represent (45) case, The negative CK14 expression is for luminal subtype (25) case (Figure 2-4).

Tab. 1. Relation CK14 expression and T stages of tumor

Stages tumor	CK14 positive expression NO.	%	ck14 Negative expression NO.	%	Total
Ta	5	26.3	14	73.7	19
T1	3	33.3	6	66.7	9
T2	34	87.2	5	12.8	39

T3	2	100	0	0	2
T4	1	100	0	0	1
Total	45	64.3	25	35.7	70

χ^2 : 26.25 df: 4 P- 0.001

Tab 2. Relation between CK14 expression and grades of tumor.

Grade Tumour	CK14 Positive expression NO.	%	CK14 Negative expression NO.	%	Total
Low grade	8	29.6	19	70.4	27
High grade	37	86	6	14	43
Total	45	64.3	25	35.7	70

χ^2 : 22.99 df: 1 P- 0.001

Tab 3. Association between CK14 expression and muscle invasive of bladder cancer

Type of tumour	CK14 positive expression NO.	%	ck14 negative expression NO.	%	Total
Muscle invasive	41	97.6	1	2.4	42
Non muscle invasive	8	28.6	20	71.4	28
Total	49	70	21	30	70

Cal. χ^2 :38.14 df: 1 P- value 0.02

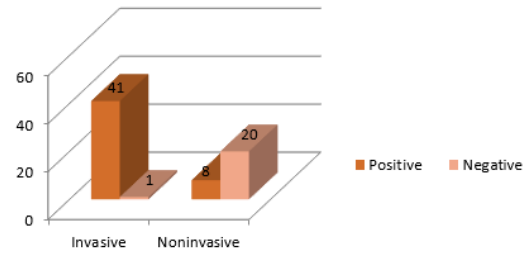


Fig 3. Correlation CK14 expression and muscle invasive of bladder cancer.

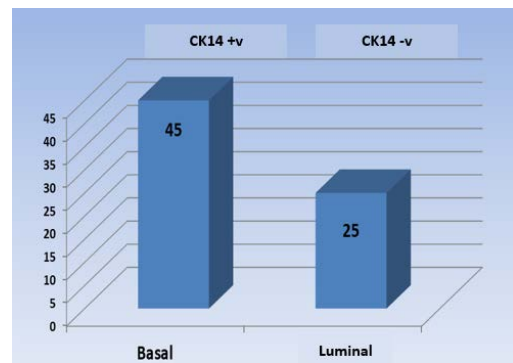


Fig 4. Subtype of bladder cancer

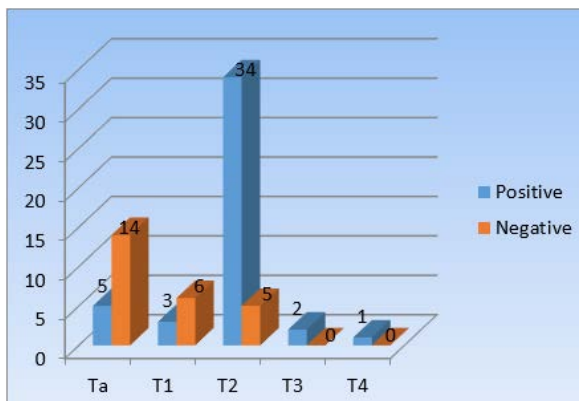


Fig 1. Represent CK14 expression, T stages of tumor

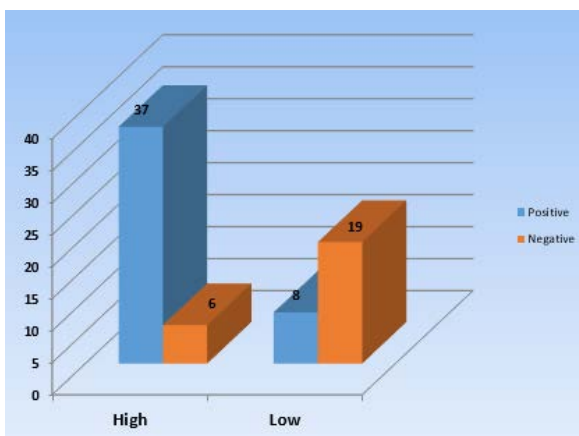


Fig 2. Relation CK14 expression and tumor grades

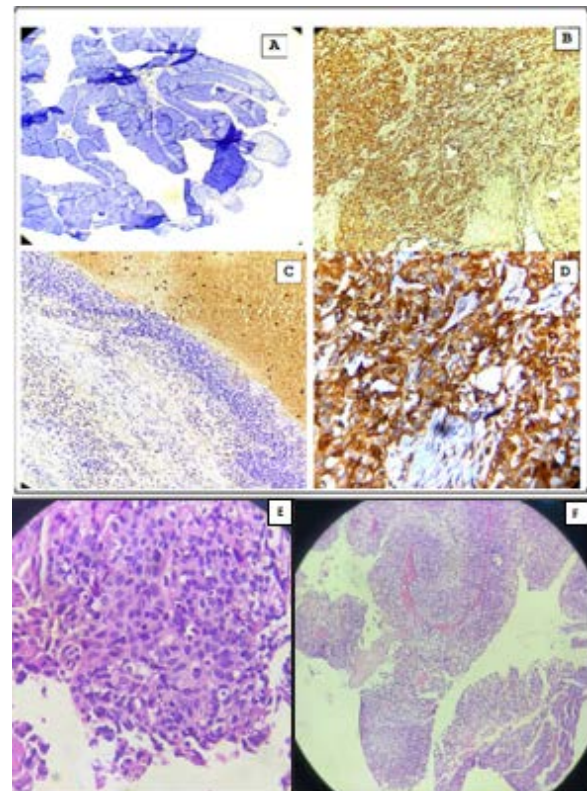


Fig 5. (A) low grade papillary urothelial carcinoma, no expression ck14(H& DAP) (10X) (B) high- grade urothelial carcinoma, show high expression of CK14(H&DAP) (40X) (C) show no expression of CK14(10X). (D) high grade urothelial carcinoma shows over expression ofCK14(H&DAP) (40X) (E) High grade urothelial carcinoma (H& E) (40X) (F) Low grade non-invasive papillary urothelial carcinoma(H&E) (40X)

DISCUSSION

In the current study the results showed high expression of CK14 in stages T2, T3, T4. The expression of CK14 is low in the Ta, T1 stages of tumor. the reason of high ck14 expression because CK14 marker of less differentiation in cancer cells, and it indicates a bad prognostication for the patient with tumor growth and metastasis, maybe due to changes in the genetic structure of cancer cells. In addition, the elevated expression of ck14 might be an indicator of the invasive tumor of the muscular layers of bladder effect and the possibility of it expanding into neighboring tissues. The result of our study agreed with the study [23]., the researcher showed the high expression of CK14 in T2 stage was (86.2%). The results of present showed that CK 14 expression was increased with a high grade of bladder cancer(86.0%) while CK14 expression showed a decrease in low grade (29.6%), It is possible the reason of high CK14 expression with a high grade is due to the fact the expression of CK14 increases with the progression of the grade bladder cancer cell, This increase in ck14 expression can be used as a marker to diagnose and determine the degree of differentiation for bladder cancer , it may be used as an indicator to expect disease behaviour in patients, This is agree with the study[24]. , the study showed the expression of CK14 increases with high grade represents (37) cases. The results of the current study revealed that Ck14 is over expressed in muscle-invasive bladder cancer whilst Ck14 expression low in non-muscle-invasive. This is due CK14 expression can be a marker of tumor invasion into the muscles of the bladder wall, increase cancer cells' metabolic activity and ability to metastases and invade. This result is agreed to the results of the researcher [25]., showed that high CK14 expression was significantly associated with muscle invasive (72.2). The results explained that positive CK14 expression expresses basal bladder cancer by (63.3%), while negative CK14 expression expresses luminal bladder cancer representing (41.4%). The reason why CK14 is associated with the basal type for bladder cancer to a high concentration of cytokeratin's related genes which include CK14. The results agreed with the study [26]., shown that CK14 expression is high in basal types and these clinical parameters are used to distinguish between luminal and basal bladder cancer and that squamous cell carcinoma is basal. The urothelial carcinoma tends to be luminal, with low expression of CK14

CONCLUSION

In conclusion, we can benefit from the excessive expression of CK14 to expect tumor behavior progression or prognosis of it, also may be of benefit to determine the type of treatment that used to treat bladder cancer. Marker is

generally used to classify bladder cancer into Luminal and basal types.

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