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A prognostic impact of interleukin 32 (IL-32) as an immune marker in patients with bladder cancer

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ABSTRACT

Background. *Urinary bladder cancer is a prevalent global health concern. IL-32 is a cytokine that promotes inflammation and has been shown in multiple studies to suppress the development of cancer cells and trigger cell death in different types of cancer cells.*

Materials and methods. *Expression of 32 interleukins was determined using an immunohistochemistry technique, which uses analysis of the exact concentrations and locations of specific compounds in tissues. This is done by using antibodies (antibodies) that react with the target protein (in this case IL-32), then identifying the sites where the active protein is located. The study included 114 diagnosed bladder cancer samples and 10 healthy controls.*

Results. *By analyzing the expression of IL-32 using IHC technology on both bladder tissue from cancer patients and healthy bladder tissue that was considered a control, the results of evaluating IL-32 levels in bladder tissue from cancer patients showed a high level.*

Conclusion. *High levels of IL-32 indicate disease progression or a poor outcome, while low levels can indicate an improvement or a good outcome. This is consistent with the fundamental aim of this study: the possibility of using IL-32 as a diagnostic or prognostic marker for disease progression and outcome prediction in cancer patients.*

Keywords: immune markers, IL-32, bladder cancers, immunohistochemistry

INTRODUCTION

Among male cancers, bladder cancer ranks second, followed by prostate cancer, while among female, it ranks ninth. Annually, about 429,793 new cases are recorded worldwide [1]. According to the Iraqi Cancer Registry (ICR), out of all the cancers diagnosed in Iraq, bladder cancer accounts for 7.01% of both male and female cases, making it the third most prevalent malignant tumor. Among men, it is the second most common tumor (11.13%), and among women, it is the ninth most common (2.97%). The highest incidence of bladder cancer was observed in Al-Muthana (11.52%) and Thi-Qar (12.24%), where it was the most common tumor, while Nineveh (3.49%) had the lowest incidence, ranking it as the tenth most common tumor [2]. Transitional cell carcinoma (TCC) is generally prevalent type, accounting for nearly 97% of cases, followed by squamous cell carcinoma (SCC) at 2% and adenocarcinoma at 1%. Bladder cancer can be categorized as invasive, extending to the deep layers of the bladder, or non-invasive, extending only to the superficial layers [3,4].

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The process by which normal urothelium transitions into bladder carcinoma is complex and multifactorial, with chronic inflammation playing a key role in initiating and advancing aggressive, invasive, and metastatic tumor growth. Although immune cells typically protect the host by inhibiting neoplastic growth, certain immune cells (including macrophages, neutrophils, and T-lymphocytes) can actually promote tumor development and progression. In bladder cancer, for example, levels of human neutrophil peptides 1, 2, and 3 increases and may encourage tumor angiogenesis and growth, while macrophages exert their effect mainly through pro-inflammatory cytokines like IL-6 and TNF-α [5].

The immune response in bladder cancer and the anti-tumor response induced by BCG are still poorly understood, despite recent progress. Immune checkpoint-directed therapy makes it all the more important to find immunological biomarkers that might predict treatment efficacy and patient outcomes. These biomarkers could include tumor-infiltrating lymphocytes (TILs) or tumor somatic mutational burden (TMB), the latter of which has prognostic importance in specific cancer contexts due to lymphocyte infiltration. Nonetheless, it appears that immune infiltration may be caused by mutations in particular pathways rather than the quantity of somatic mutations, according to data in bladder cancer. There is an increasing amount of evidence suggests that the ubiquitous myeloid and innate populations within tumors may be responsible for dampening tumor immunosurveillance. There is, therefore, a continuing effort to find better biomarkers that include the latest findings in tumor immunology [5,6].

IL-32, a pro-inflammatory cytokine expressed by various immune and non-immune cells, has been shown to play an important part in cancer, inflammation, and infections. Although the function of IL-32 is still not completely clear, recent studies suggest that it may be involved in both the intracellular and secretory pathways and may be a useful biomarker for predicting the progression or recurrence of bladder cancer. NK cells, macrophages, monocytes, and T lymphocytes are among the human immune cells that express IL-32. Non-immune cells that do the same include hepatocytes, epithelial cells, fibroblasts, endothelial cells, and mesenchymal stromal cells. It is also seen in cells that have cancer, such as those that have melanoma, colon, pancreatic, or thyroid.

An essential source of IL-32 in a number of disorders has been identified as regulatory T cells (Tregs). An increase or induction of IL-32 expression is normally observed in response to infections, pro-inflammatory cytokines, and various forms of cellular stress, like hypoxia, although it can be expressed under basal conditions as well [7].

MATERIALS AND METHODS

Study design

The research was conducted at the Cancer Research Unit located within the College of Medicine at Thi-Qar University. It was a cross-sectional descriptive study that was done at the hospital level. To gather archival paraffin blocks from individuals diagnosed with bladder cancer, a basic random sample method was employed. However, ten samples of normal bladder tissue, in addition to the reports attached to the sample, were obtained from private laboratories for conducting histological analyzes in the city of Nasiriyah. Note that these samples are biopsies that were suspected to be cancer or a benign tumor, but the final diagnosis showed that they are normal tissue. This is one of the reasons for the small number of control samples in this study.

Based on their pathology reports, 114 patients were included in this cross-sectional study that examined bladder cancer. All adult male and female patients referred to the Histopathology Unit at Al-Hussein Teaching Hospital or private laboratories between January 1, 2021 and April 30, 2022, who have confirmed bladder cancer by biopsy or surgical report, were eligible to participate. Exclusion criteria included benign conditions and records with unclear data. The specimens were extracted from archival materials that had been formalin-fixed and embedded in paraffin. Their ages varied from 23 to 98 years. After urologists made their initial diagnoses, histopathology verified the conditions for the patients. The majority of patients did not receive preoperative radiotherapy or chemotherapy. The histopathology reports included information on tumor morphology, type, muscle invasion, and grade. Each block was used to extract sections that were 5µm-3µm thick. These sections were then placed on positively charged slides and subjected to immunohistochemistry (IHC) staining. The intensity of the staining was used to classify the sections into four grades: 0 (no staining), 1 (weak staining), 2 (mid staining), and 3 (strong staining) [8].

Statistical Analysis

Version 26 of the Statistical Package for the Social Sciences (SPSS) was utilized to conduct the statistical analysis. To compare the groups statistically, we utilized the chi-square test; a 0.05 P value was deemed significant [8].

RESULTS

Immunohistochemical scoring system

Two separate pathologists used a semi-quantitative scoring system to determine the level of IL32 staining in the cytoplasm and nucleus in all tissue sections. The

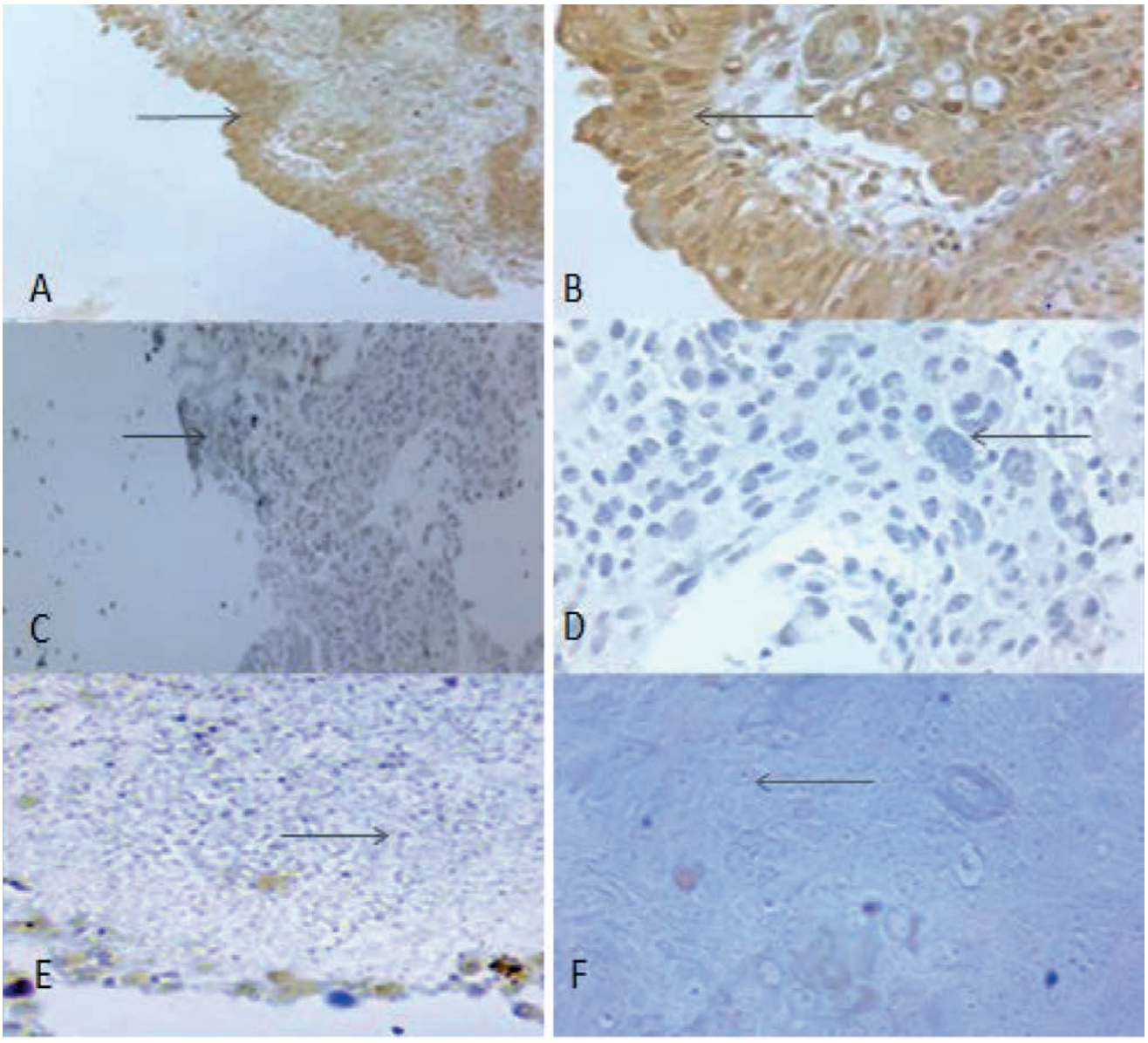

FIGURE 1. *Immunohistochemical staining for IL-32 in bladder cancer tissues. A-B. A high-grade papillary urinary bladder cancer show IL-32 staining was strong (magnification, 10×-40×). C-D. A negative control for IL-32 expression in bladder cancer tissues is shown (magnification, 10×-40×). E-F. IL-32 staining was weak in noncancerous bladder tissue (normal bladder tissue) (magnification, 10×-40×)*

scoring relied on the overall intensity of staining and was given one of four possible levels: no expression, mild expression, moderate expression, and strong expression. The levels of IL32 expression were determined by the percentage of stained cells: no cells express the stain, 1-25% of cells express it, 26-50% of cells express it, 51-75% of cells express it or more than 76%.

Association between sex, age, cancer invasion ability with cancer characteristics

Based on the study's findings, the correlation between sex and cancer kinds is not statistically significant (p. value <0.05). There is also non-statistically significant relationship between age and type of bladder cancer at p. value <0.05. The situation did not differ for the sex with grad or for the sex with stage, which is the absence of a statistically significant relationship, as the p. value for them were at p. value <0.05 respectively. In contrast to the paragraph above, the statistical results of the data demonstrated that there were statistically significant disparities among the two cases (invasion and non-invasion) and the type of bladder cancer were at p <0.05 (Table 1).

Statistical results of immunohistochemical for expression IL32

IL-32 Expression by immunohistochemical in Cancer Patients and Control Group

Their study recorded a significant superfat of IL-32 expression in patient's cancer cell cytoplasm, nucleus, and total cell compared with control group at p<0.05, (Table 2).

TABLE 1. *Statistical values of urinary bladder cancers data from patient reports*

Cancer type/ Sex	Male		Female		Total	
	No.	%	No.	%	No.	%
Adenocarcinoma	1	1.2	0	0.0	1	0.9
Papillary Tcc	30	35.3	10	34.5	40	35.1
Squamous cell car	7	8.2	6	20.7	13	11.4
Tcc	47	55.3	13	44.8	60	52.6
Total	85	74.6	29	25.4	114	100
p. value 0.291						

Cancer type/ Age	<40		41-55		56-70		>70		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Adenocarcinoma	0	0.0	1	100	0	0.0	0	0.0	1	0.9
Papillary Tcc	2	5.0	10	25.0	17	42.5	11	27.5	40	35.1
Squamous cell car	1	7.7	2	15.4	6	46.2	4	30.8	13	11.4
Tcc	1	1.7	10	16.7	24	40.0	25	41.7	60	52.6
Total	4	3.5	23	20.2	47	41.2	40	35.1	114	100
p. value 0.535										

Grade/ Sex	Male		Female		Total	
	No.	%	No.	%	No.	%
High	47	55.3	20	69.0	67	58.8
Med	0	0.0	1	3.4	1	0.9
Low	38	44.7	8	27.6	46	40.3
Total	85	74.6	29	25.4	114	100
p. value 0.075						

Stage/ Sex	Male		Female		Total	
	No.	%	No.	%	No.	%
Non	47	55.3	19	65.5	66	57.9
T1	14	16.5	0	0.0	14	12.3
T2	13	15.3	5	17.2	18	15.7
T2a	4	4.7	2	6.9	6	5.3
T2b	3	3.5	2	6.9	5	4.4
T3	1	1.2	0	0.0	1	0.9
T4	3	3.5	1	3.4	4	3.5
Total	85	74.6	29	25.4	114	100
p. value 0.389						

Cancer type / Invasion Ability	Invasion		Non-invasion		Total	
	No.	%	No.	%	No.	%
Adenocarcinoma	1	100	0	0.0	1	0.9
Papillary Tcc	4	10.0	36	90.0	40	35.1
Squamous cell car	6	46.2	7	53.8	13	11.4
Tcc	24	40.0	36	60.0	60	52.6
Total	35	30.7	79	69.3	114	100
p. value 0.003						

TABLE 2. *Association between IL-32 expression in patients and control*

Expression sources/ patients-control	Patients No. 114	Control No. 10	p. value
	Mean±S. D		
Cytoplasm	4.79±0.69	2.60±0.60	<0.001
Nucleus	2.25±1.48	0.50±0.70	<0.001
Cell	3.52±0.86	1.55±0.86	<0.001

Association between IL-32 expression and sex data from patient reports

Examining the correlation between sex and IL-32 expression allowed us to learn whether sex influenced IL-32 expression in bladder cancer (in cytoplasm, nucleus, and cell). There is no statistically significant link between sex and IL-32, as shown in Table 3, with p-values less than 0.05 for the cytoplasm, nucleus, and cell.

TABLE 3. *Correlation between the expressions of IL-32 in bladder cancer tissue based on the results of the nucleus, cytoplasm, and cell as a whole and sex data*

Expression sources/Sex	Female No. 29	Male No. 85	p. value
	Mean ± S. D		
Cytoplasm	4.82±0.49	4.78±0.75	0.805
Nucleus	2.23±1.48	2.26±1.49	0.928
Cell	3.52±0.78	3.52±0.89	0.983

Association between IL-32 expression and Age Groups in years from patient reports: The correlation between age and IL-32 cytoplasmic, nuclear, and cell-level expression in bladder cancer was the subject of another statistical investigation. The statistical results revealed that there is no statistically significant link in the cytoplasm (p. value = 0.208), nucleus (p. value = 0.237), and cell (p. value = 0.100). Comparing the second and fourth age groups for IL-32 cytoplasmic expression, we find that there are statistically significant differences (p = 0.0362-4 for LSD p. value) and, p = 0.0172-4 for IL-32 cell expression. (Table 4)

TABLE 4. *Linkage between the expressions of IL-32 in bladder cancer tissue based on the results of the nucleus, cytoplasm, and cell as a whole and age groups in years*

Age groups/ Expression sources	Cases No.	Cytoplasm	Nucleus	Cell
		Mean ± S. D		
<40	4	4.70±0.70ab	2.60±0.43	3.65±0.38ab
41-55	23	5.05±0.40a	2.58±1.51	3.81±0.80a
56-70	47	4.77±0.75ab	2.38±1.54	3.58±0.92ab
>70	40	4.67±0.72b	1.88±1.42	3.27±0.82b
ANOVA p. value LSD p. value		0.208 0.0362^{-4}	0.237	0.100 0.0172^{-4}

Association between IL-32 expression and type of bladder cancer from patient reports

In addition, the scanning for how different types of bladder cancer were associated with different levels of IL-32 expression in the cytoplasm, nucleus, and cell. Our statistical analysis revealed that the cytoplasm had an ANOVA p. value of 0.036, the nucleus had an ANOVA p. value of <0.001, and the cell had an ANOVA p. value of <0.001. This suggests that there is a statistically significant relationship between the cytoplasm and IL-32 expression in Papillary Tcc and Squamous cell carcinoma, but things are different when it comes to the nucleus and cell. These results are shown in Table 5.

Association between IL-32 expression and grade of cancer from patient reports

The statistical significances in the case of grade were somewhat different, as the relationship between IL-32 expression in the cytoplasm, nucleus, and cell with the grade was evaluated, anyway the statistical data showed that p. value 0.196, 0.041, and 0.220 in the cytoplasm, nucleus, and cell, respectively. This indicates a statistically significant relationship with regard to the expression of IL-32 in the nucleus, but the case differs with the cytoplasm and cell, which showed results indicating non-significant differences in IL-32 expression. (Table 6)

TABLE 5. *linkage between the expressions of IL-32 in bladder cancer tissue according to the results of the nucleus, cytoplasm, and cell as a whole and Type of Bladder Cancer*

Type of bladder cancer/ Expression sources	Cases No.	Cytoplasm	Nucleus	Cell
		Mean ± S. D		
Papillary Tcc	40	4.96±0.59a	2.87±1.27a	3.91±0.66a
Squamous cell car	13	4.41±1.03b	1.12±1.13c	2.76±0.86c
Tcc	60	4.74±0.63ab	2.13±1.50b	3.43±0.86b
ANOVA p. value		0.036	<0.001	<0.001

TABLE 6. *Relation between the expressions of IL-32 in bladder cancer tissue according to the results of the nucleus, cytoplasm, and cell as a whole and grade of cancer*

Expression sources /Grade	High No. 67	Low No. 46	p. value
	Mean ± S. D		
Cytoplasm	4.87±0.58	4.70±0.81	0.196
Nucleus	2.04±1.36	2.61±1.57	0.041
Cell	3.45±0.75	3.65±0.98	0.220

Association between IL-32 expression and stages of cancer from patient reports

By analyzing statistical data derived from the ANOVA p values of 0.709, <0.001, and 0.001 in the cytoplasm, nucleus, and cell, respectively, we can determine the penultimate criterion, which pertains to the progression of bladder cancer and its correlation with IL-32 expression in the cells. The data presented in Table 7 demonstrate the presence of statistically significant variations in the expression of IL-32 in both the nucleus and the cell as a whole, in contrast to the cytoplasm, where no such relationship is observed.

Association between IL-32 expression and invasion plus non invasion of cancer from patient reports

One of the important data that was recorded in this study is the presence of invasion and the non-invasion of bladder cancer and its relationship with the expression of interleukin 32 in the nucleus, cytoplasm and the cell as a whole as well, which became clear after completing the statistical study based on the p value 0.590, <0.001, and <0.001 in the cytoplasm, nucleus, and cell, respectively. There is no significant or statistically significant value regarding the expression of IL-32 in the

TABLE 7. *Relation amongst the expressions of IL-32 in bladder cancer tissue and Stages of Cancer according to the results of the nucleus, cytoplasm, and cell as a whole*

Stages/ Expression sources	Cases No.	Cytoplasm	Nucleus	Cell
		Mean ± S. D		
Non	66	4.74±0.70	2.79±1.47a	3.76±0.89a
T1	14	4.88±0.51	2.51±1.47ab	3.70±0.86a
T2	18	4.80±0.76	0.92±0.66d	2.86±0.55b
T2a	6	5.13±0.53	1.56±0.70b	3.35±0.22ab
T2b	5	4.96±0.45	1.08±0.59cd	3.02±0.22b
T4	4	5.05±0.50	1.35±0.94c	3.20±0.32ab
ANOVA p. value		0.709	< 0.001	0.001

cytoplasm, but the significant values differ with the nucleus and the cell as a whole, which showed results indicating the presence of significant differences statistical or significant in expression of IL-32. (Table 8)

TABLE 8. *Attachment amongst the expressions of IL-32 in bladder cancer tissue and invasion plus non invasion of cancer according to the results of the nucleus, cytoplasm, and cell as a whole*

Expression sources/ invasion plus non-invasion	Invasion No. 34	Non No. 80	p. value
	Mean ± S. D		
Cytoplasm	4.84±0.75	4.77±0.67	0.590
Nucleus	1.11±0.67	2.74±1.46	<0.001
Cell	2.97±0.51	3.75±0.88	<0.001

DISCUSSION

Realistically, prognostic markers predict "the natural course of an individual cancer, distinguishing good outcome tumors from poor outcome tumors, and they guide the decision of whom to treat and/or how aggressively treated" when a patient is first diagnosed with cancer and their prognosis can vary greatly, prognostic markers become extremely crucial. Still, "no prognostic marker can accurately predict outcome for an individual patient; it provides a probability estimate of outcome for a heterogeneous population of patients," as pointed out by Duffy et al. [9]. It is important to note that prognostic markers could play a pivotal role in determining whether patients with mild cancer are over treated, resulting in fewer adverse effects from adjuvant systemic therapies, or whether patients with aggressive and potentially fatal cancer are undertreated, preventing them from receiving the best possible combination of local and systemic treatments [9]. Even though there have been hundreds of proposals for predictive biomarkers in recent years, very few have made it to the clinical trials stage. Staging is the gold standard when it comes to bladder cancer, both for diagnosis and therapy planning [10,11].

IL-2-activated NK-cells and mitogen-activated T-cells are the ones responsible for producing IL-32, which was earlier referred to as NK4 [12]. The initial group to document NK4 in 1992 was Dahl et al. [13], who postulated that NK4 was an unrelated new product of activated NK and T cells. Six isoforms, IL-32α, IL-32β, IL-32γ, IL-32δ, IL-32ε, and IL-32ξ, have been found at the IL-32 encoding gene locus, which is situated on chromosome 16p13.3. When this research first began, IL-32 expression in inflammatory trouble like rheumatoid arthritis, tuberculosis, and inflammatory bowel disease was the main emphasis [14,15]. It has also been shown that, under specific circumstances, the new pro-inflammatory cytokine IL-32 can either promote tumor growth or inhibit it [16]. Patients with stomach cancer had higher than normal levels of serum IL-32, and ELISA and immunohistochemistry both showed that a K-562 lymphoblastic cell line released more IL-32 than normal [17].

Overall, the current study's analyses pointed out that normal bladder tissue had weak positive staining, and the staining intensity was significantly high or strong in bladder cancer tissue so this study is fully consistent with Nishida et al. [18] and Takagi et al. [8] whose found IL-32 expression in pancreatic cancer (cell lines) and human pancreatic tissue, and who demonstrated that straight pancreatic duct cells showed limited confirmation staining, but that chronic pancreatitis cells showed significantly stronger staining and pancreatic cancer cells showed strong staining. Similarly, in their study of IL-32 expression in human lung cancer, Sorrentino et al. [19] demonstrated that IL-32 is highly reactive in malignant tumors when stained with immunohistochemistry. The authors also emphasized the link between inflammation and lung cancer, implying that IL-32, a proinflammatory cytokine, plays a role in the enlargement of lung cancer. But no one has looked at the clinical-pathological relevance of IL-32 expression in bladder cancer before. Within this research analysis, it is observed Men have a fourfold higher probability of developing urinary bladder cancer compared to women. This finding aligns with the data presented in the Cancer Statistics 2019 report, which states that the ratio of males to females for this specific type of cancer varies between 1:3 and 1:5 globally [20]. In contrast, Jordan recorded a male-to-female ratio of 1:9, indicating a higher number of males compared to females [21]. Furthermore, this investigation, when taken in conjunction with global data, consistently shows a significant prevalence of males in all types of urinary bladder cancer. The reason for this pattern can be related to the greater probability of males engaging in agricultural and industrial occupations, which can increase their exposure to cancer-causing chemicals [22]. A large proportion of urinary bladder cancer instances (over 70%) occur in patients aged 50 and over, while less than 30%

occur in younger adults. This fits in with a lot of findings presented by Bilim et al. [23] from Japan. Cancer rates tend to rise with age, and there are a lot of hypotheses and expectations that try to explain this trend. For example, it may simply take time for cancer to develop, or it may be that specific cells are more vulnerable to substances that cause cancer as they age. The immunological system's vigilance in detecting a single aberrant cancer cell may deteriorate with age. As cells age, they may find it more challenging to regulate their own proliferation. A cell's capacity to repair DNA inevitably declines with age, which would explain the phenomenon.

The result of our study shows that the proportion of patients with low-grade tumor (approximately 40%), in addition to the muscle invasion seen in cases, is mostly high grade, and this is consistent with many studies conducted in Iraq [24-27]. Possible explanations include: high-grade tumors are more likely to invade muscle tissue than low-grade tumors; high-grade tumors have genetic and molecular abnormalities that make them aggressive; sample size is an issue; histological grading of bladder cancer is subjective and has its limitations, especially when applied to biopsy material; neoplasm characteristics can vary from region to region; and finally, a cystoscopy biopsy may reveal a low-grade cancer, whereas a surgical specimen may indicate a more severe condition. Our study revealed that 55.30% of urinary bladder cancer masses were identified as high-grade, whereas 44.70% were classed as low-grade. This pattern is similar to a study carried out in Nepal, which reported that over half of the cases (52.2%) were categorized as high-grade. El-Siddig et al. [28] found the opposite to be true; they classified two-thirds of the instances as low grade and one-third as high grade.

Current study reveals, that tumor stages t1 and t2 were the most commonly observed. Additionally, a separate study found that the majority of cases of urinary bladder cancer (75-80%) occurred without infiltration of the bladder muscle wall. Non-invasive cancers can be effectively treated using a telescopic method called transurethral resection of bladder tumor (TURBT). This procedure involves removing the cancerous tissue and then administering chemotherapy or vaccine-based therapy straight into the bladder [29,30]. There are a few reasons why T1 and T2 stages are frequently encountered in bladder cancer one of it early detection that mean T1 bladder cancer is often detected at an early stage because it may cause hematuria. Moreover, T1 tumors can sometimes be identified during routine screenings, such as cystoscopy or imaging. However, bladder cancer T1 has the potential to progress to T2 or higher stages. This progression can occur over time as the tumor grows and invades deeper layers of the bladder wall. Thus, some T1 tumors may advance to T2 stage if not treated promptly. It is crucial to acknowledge that the prevalence of different tumor stages can vary based on various factors, including the population studied, geographic location, and advancements in diagnostic techniques.

Wu et al. [31] used single-cell sequencing data to show that IL-32 expression in bladder cancer tissues is typically high in malignant cases, indicating a large level of expression and this is consistent with the current study that recorded a significant increase of IL-32 expression in patient's cancer cell cytoplasm, nucleus, and total cell compared with control group at p. value < 0.05, as in (Table 2). Increased severity of stomach inflammations, gastric cancer, bladder cancer, and chronic rhino sinusitis is associated with interleukin [IL]-32, a critical modulator in the pathophysiology of numerous clinical issues primarily generated by interleukin [IL]-8. In addition to playing a role in inflammation development, it triggers the production of a series of powerful inflammatory cytokines [12,31]. As reported, there are a number of possible roles for IL-32 [32]. In order to investigate IL-32's role in bladder cancer in more detail, Wu et al. [31] attempted to replicate the impact of IL-32 secreted by Tregs cells on bladder cancer cells by incubating these cells with a low quantity of the cytokine. T24 and EJ cell movement was accelerated by IL-32, which is consistent with its effect on colorectal cancer [33]. Furthermore, IL-32 enhanced bladder cancer cell invasion. In order to understand how IL-32 interpose in invasion and migration, researchers looked at the databases of The Cancer Genome Atlas Program (TCGA) and The Genotype Tissue Expression Project (GTEx). They discovered that IL-32 expression was linked to the abundance of the C-C motif chemokine ligand 4 (CCL4), which meant that IL-32 enhanced bladder cancer metastasis [31,34]. Therefore, there is agreement with the data recorded in this study which included presence of invasion and the non-invasion of bladder cancer and its relationship with the expression of interleukin 32, which showed results indicating the presence of significant differences statistical or significant in expression of IL-32 as in Table 8.

As can be seen from Table 3, there is no statistically significant relationship between IL-32 expression and either sex or age, as indicated by the lack of an association between the two variables. This means that IL-32 is not affected by either sex or age. Data from Table 4 show that there were statistically significant differences in IL-32 cytoplasmic and nuclear expression between the second and fourth age groups, with an LSD p. value of 0.0362-4 and a p. value of 0.0172-4, respectively. Jiang et al. [35] statistical data did not differ from the present study with regard to age, gender, and their relationship to IL-32, which addressed the issue of cytokines as biomarkers for the diagnosis of interstitial cystitis/bladder pain syndrome and mapping their clinical characteristics.

The other aspect involved evaluating the relationship between the expression of IL-32 in the cytoplasm, nucleus, and cell with the type of bladder cancer, the statistical data showed that ANOVA p. value < 0.05, for cytoplasm, nucleus, and cell, respectively, this indicates that there is a statistically significant relationship with respect to the expression of IL-32 as in (Table 5). IL-32 expression can be influenced by multiple factors, such as genetic alterations, epigenetic modifications, and interactions with the tumor microenvironment. However, the specific mechanisms underlying the differences in IL-32 expression patterns among bladder cancer subtypes are not well understood. Also, different subtypes of bladder cancer have different genetic and molecular characteristics, which may explain why IL-32 expression varies across these subtypes. To better understand the impact of IL-32 expression in various bladder cancers and the molecular mechanisms at activities, additional researches are required [7].

The statistical significances in the case of grade were somewhat different, as the relationship between IL-32 expression in the cytoplasm, nucleus, and cell with the grade was evaluated, anyway the statistical data showed that p. value 0.196, 0.041, and 0.220 in the cytoplasm, nucleus, and cell, respectively. This indicates a statistically significant relationship with regard to the expression of IL-32 in the nucleus, but the case differs with the cytoplasm and cell, which showed results indicating non-significant differences in IL-32 expression as in Table 6. IL-32 is primarily known as a cytoplasmic protein involved in pro-inflammatory responses. However, there is evidence suggesting that IL-32 can translocate to the nucleus under certain conditions. These reasons for the difference in localization of IL-32 in the nucleus rather than the cytoplasm in the case of bladder cancer may be caused by protein-protein interactions, IL-32 has been reported to interact with several nuclear proteins, such as histones and transcription factors. These interactions may influence the nuclear translocation of IL-32 in bladder cancer cells or genetic alterations or epigenetic modifications specific to bladder cancer cells could influence IL-32 localization also it is important to note that the subcellular localization of IL-32 in bladder cancer may vary between different individuals or even within different stages of the disease [36].

Association between IL-32 expression and Stages of Cancer from patient reports, there is no statistically significant relationship with regard to the expression of IL-32 in the cytoplasm, but the situation differs with the nucleus and the cell as a whole, which showed results indicating the presence of statistically significant differences in the expression of IL-32 in both nucleus and cell as in Table 7. The localization of IL-32 within the cell can vary depending on the cellular context and disease state. In the case of bladder cancer, studies have shown that there is an increased localization of IL-32 in the nucleus compared to the cytoplasm at different stages of the disease. The exact reasons for this nuclear localization of IL-32 in bladder cancer may involve complex interactions between IL-32 and other cellular components. However, there are a few possible explanations that IL-32 has various isoforms resulting from alternative splicing, and different isoforms may have different subcellular localizations. It is possible that specific isoforms of IL-32 found in bladder cancer cells have a greater tendency to accumulate within the nucleus or may explanations by disease-related alterations that involve bladder cancer is associated with genetic and epigenetic alterations that can affect various cellular processes. It is possible that these alterations directly or indirectly influence the localization of IL-32 within the cell [36-38]. It important to note that the understanding of IL-32 and its role in bladder cancer is still evolving, and further research is needed to fully elucidate the mechanisms underlying its nuclear localization in different stages of the disease.

CONCLUSION

The results of evaluating IL-32 levels in bladder tissue from cancer patients showed a high level compared to the healthy bladder tissue used as a control. This suggests that high levels of IL-32 indicate disease progression or a poor outcome, while low levels can indicate an improvement or a good outcome. These findings are consistent with the fundamental aim of this study, to explore the possibility of using IL-32 as a diagnostic or prognostic marker for disease progression and outcome prediction in cancer patients.

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Ethical consideration:
The research followed the scientific and ethical research guidelines outlined in the Declaration of Helsinki. Prior to the start of the study, approval was obtained from the Ethics Committee of the College of Medicine and Science at Thi-Qar University (No. 909/2022 and 960/2022) and additionally from the Department of Health in Thi-Qar (No. 655/2022) and the Ministry of Health (No. 02/2021).

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