

Association of Leptin Receptor Expression with Prognostic Pathological Features in Sudanese Urinary Bladder Carcinoma

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Abstract

Bladder carcinoma shows marked pathological heterogeneity, and tissue-based markers may help identify tumors with aggressive pathological features. Data on molecular pathology markers in bladder carcinoma are still limited in African cohorts, including Sudan. This study assessed whether leptin receptor (LEPR) immunohistochemical expression was associated with histological grade, pathological pT category, muscle invasion, and lymphovascular invasion in Sudanese bladder carcinoma. LEPR expression was assessed by immunohistochemistry in 153 archived FFPE urinary bladder carcinoma specimens from

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four Sudanese institutions and was classified using the immunoreactive score. Urothelial carcinoma predominated in this cohort (136/153, 88.9%). High-grade disease was present in 49/136 urothelial carcinomas (36.0%). Pathological pT category was assessable in 149 cases, with 54/149 tumors (36.2%) classified as pT2 or higher. Muscle invasion was present in 54/149 tumors (36.2%), and lymphovascular invasion was present in 56/149 tumors (37.6%). Moderate LEPR immunoreactivity was more frequent in high-grade than low-grade urothelial carcinomas (28.6% vs 10.3%; $p = 0.002$). LEPR expression also varied by pT category ($p = 0.002$), muscle invasion ($p = 0.001$), and lymphovascular invasion ($p = 0.001$). Moderate expression was most frequent in pT2-or-higher tumors (35.2%) and was higher in muscle-invasive than non-muscle-invasive tumors (35.2% vs 18.9%). In this Sudanese cohort, higher LEPR expression was observed frequently in tumors with adverse pathological features. Further studies should include matched normal urothelium, outcome data, and adjusted analyses to clarify whether LEPR has biological and prognostic significance.

Keywords

Bladder Carcinoma, Leptin Receptor, Immunohistochemistry, Tumor Aggressiveness, Sudan

1. Introduction

Urinary bladder carcinoma remains a major cause of cancer-associated morbidity and mortality worldwide. It shows clear sex differences and includes non-muscle-invasive and muscle-invasive forms, which differ in biology, treatment, and outcome [1]. Tobacco exposure, occupational carcinogens, and other environmental factors remain major contributors to disease burden [2] [3]. In areas where chronic inflammatory exposures are common, including schistosomiasis-endemic regions, the histological pattern and clinical profile may differ from those reported in many high-income settings [3].

Pathological assessment remains the basis of clinical risk stratification in bladder cancer. Tumor grade, pathological stage, muscularis propria invasion, and lymphovascular invasion are central markers of aggressive disease and guide treatment decisions [4]-[6]. However, these features do not fully explain variation in tumor behavior. This has increased interest in tissue-based biomarkers that may refine pathological assessment, especially in settings where molecular data are limited.

The leptin receptor (LEPR; Ob-R) is a class I cytokine receptor. Through binding to leptin, LEPR can activate signaling pathways involved in cell survival, proliferation, inflammation, invasion, and angiogenesis, including JAK2/STAT3, PI3K/Akt, and MAPK pathways [7] [8]. In several malignancies, dysregulated leptin-LEPR signaling has been linked to tumor growth, metastatic behavior, and resistance to therapy [7] [8]. These observations support a biologically plausible role

for LEPR in aggressive tumor phenotypes, although this role appears to be tissue-specific and context-dependent.

Evidence in bladder and urothelial carcinoma remains limited but relevant. In urinary bladder carcinoma, leptin expression has been associated with advanced stage, muscularis propria invasion, vascular invasion, nodal involvement, metastasis, and poorer survival [9]. Another bladder cancer study reported higher expression of adipocytokines and their receptors in bladder tumors than in benign urothelial tissues, with leptin-related expression linked to progression in muscle-invasive disease [10]. In upper tract urothelial carcinoma, LEPR overexpression has also been associated with poorer recurrence-free and cancer-specific survival [11]. Together, these findings suggest that leptin-LEPR signaling may be related to aggressive urothelial tumor behavior.

Data from Sudan remain limited. Available Sudanese studies have mainly described the clinical and pathological profile of bladder cancer, with little evaluation of tissue-based molecular markers such as LEPR [12]. This lack of molecular pathology data is important because genetic background, environmental exposure, infection-related inflammation, and patterns of clinical presentation may differ across populations. Region-specific data are therefore needed to evaluate the relevance of LEPR expression in Sudanese bladder carcinoma. This study aimed to evaluate LEPR immunohistochemical expression in archived formalin-fixed, paraffin-embedded (FFPE) urinary bladder carcinoma tissues from Sudanese patients. It assessed the association between LEPR expression and established pathological indicators of tumor aggressiveness, including tumor grade, pathological stage, muscular invasion, and lymphovascular invasion. The study was designed to assess pathological association rather than mechanism or independent prognosis.

2. Materials and Methods

2.1. Study Design and Setting

This retrospective, cross-sectional, multicenter tissue-based study used archived formalin-fixed, paraffin-embedded (FFPE) urinary bladder carcinoma specimens from Sudanese patients. The study evaluated immunohistochemical expression of leptin receptor (LEPR; Ob-R) and its association with established pathological indicators of tumor aggressiveness. Cases were obtained from four pathology centers in Sudan: Ibn Sina Hospital, Omdurman Military Hospital, Atbara Medical Complex, and El-Mak Nimr University Hospital. These centers were included because they had suitable archived bladder tumor material and sufficient case numbers. Sample collection was conducted between September 2022 and March 2025, and archived cases diagnosed from 2015 onward were eligible.

2.2. Case Selection

The study population comprised Sudanese patients with histologically confirmed urinary bladder carcinoma and an available representative FFPE tumor block. All

histological subtypes were eligible, and no restrictions were applied by age or sex. Cases were included when the archived tissue was adequately preserved for histopathological review and LEPR immunohistochemical staining. Cases were excluded when poor fixation, insufficient tumor tissue, extensive necrosis, or poor tissue preservation could compromise histopathological or immunohistochemical assessment. The final study cohort included 153 FFPE urinary bladder carcinoma cases that met the eligibility and tissue-quality criteria.

2.3. Tissue Sectioning and Specimen Workflow

For each FFPE block, tissue sections were prepared using a rotary microtome. One 3-micrometer section was mounted on an uncharged glass slide for routine hematoxylin and eosin (H&E) staining. One additional 4-micrometer section was mounted on a positively charged poly-L-lysine-coated glass slide for LEPR immunohistochemistry. To minimize cross-contamination, instruments and work surfaces were cleaned between cases and consumables were changed as needed.

2.4. H&E Staining and Histopathological Assessment

H&E staining was performed on 3-micrometer sections using an automated Leica ST5020 staining system. Sections were deparaffinized, rehydrated through graded ethanol, stained with modified Harris hematoxylin, blued with Scott's tap water substitute, counterstained with eosin, dehydrated, cleared, and mounted according to routine laboratory procedures.

All H&E-stained sections were reviewed by an experienced pathologist using bright-field light microscopy. Histopathological review confirmed the diagnosis and assessed histological subtype, tumor grade, depth of invasion, muscularis propria involvement, and lymphovascular invasion. Histological grading was performed according to the 2022 World Health Organization classification of urinary and male genital tumors [4]. Pathological stage was assigned using the tumor component of the eighth edition American Joint Committee on Cancer TNM system [5]. Because staging was based on transurethral resection of bladder tumor specimens, tumors were categorized as carcinoma in situ (Tis), non-invasive papillary carcinoma (Ta), lamina propria-invasive carcinoma (T1), or T2 or higher. Further separation of T3 and T4 disease was not attempted because TURBT specimens do not reliably assess extravesical extension. The main pathological endpoints were tumor grade, pathological stage, muscular invasion, and lymphovascular invasion. Because survival and follow-up data were not available, these variables were analyzed as pathological indicators of tumor aggressiveness rather than direct survival outcomes.

2.5. LEPR Immunohistochemistry

LEPR immunohistochemical staining was performed on 4-micrometer FFPE sections using a Leica BOND-MAX automated immunostainer and the Leica BOND

Polymer Refine Detection Kit. Sections underwent automated deparaffinization and rehydration with BOND Dewax Solution, followed by washing with BOND Wash Solution. Heat-induced antigen retrieval was performed with Bond Epitope Retrieval Solution 1, a citrate-based buffer at pH 6.0, at 97°C for 20 minutes, followed by cooling at room temperature. Endogenous peroxidase activity was blocked using the peroxide block reagent supplied with the detection kit. A protein-based, serum-free blocking reagent was applied to reduce nonspecific binding.

Sections were incubated with rabbit polyclonal anti-LEPR antibody (GeneTex, GTX37636) at a dilution of 1:300 for 30 minutes at room temperature. The antibody was diluted with Bond Primary Antibody Diluent. Human placental tissue was used as an external positive control. Negative controls were prepared by replacing the primary antibody with antibody diluent. Immunoreactivity was visualized with 3,3'-diaminobenzidine, producing a brown reaction product at sites of antigen expression. Sections were counterstained with Harris hematoxylin, dehydrated, cleared, mounted, and examined by bright-field microscopy.

2.6. Immunohistochemical Scoring

LEPR expression was assessed semi-quantitatively using the German immunoreactive score (IRS) system [13]-[15]. Tumor-cell membranous and/or cytoplasmic staining was evaluated. For each case, one representative tumor-containing section was selected after review of available tissue. Each slide was first scanned at low magnification to assess staining distribution, followed by evaluation of five representative viable high-power fields at 400× magnification. Areas with necrosis, crush artifact, hemorrhage, or edge artifact were excluded. A minimum of 200 tumor cells was assessed per case.

The IRS was calculated by multiplying the percentage score by the staining-intensity score. The percentage of positive tumor cells was scored as 0 for no staining, 1 for less than 10%, 2 for 10% - 50%, 3 for 51% - 80%, and 4 for more than 80%. Staining intensity was scored as 0 for negative, 1 for weak, 2 for moderate, and 3 for strong staining. The final IRS ranged from 0 to 12 and was classified as negative (0), weak positive (1 - 3), moderate positive (4 - 8), or strong positive (9 - 12). These descriptive categories were derived directly from the IRS categories and were not treated as a separate scoring system. All stained slides were evaluated by a single experienced observer who was blinded to clinicopathological data, except for the histological diagnosis. To assess scoring reproducibility, 23 randomly selected cases were re-evaluated after a defined interval.

2.7. Data Collection and Analytical Variables

Clinical and pathological data, including age and sex, were extracted from hospital records and pathology reports. Immunohistochemical results were entered into a structured database for statistical analysis. LEPR expression was analyzed using the IRS-derived categories of negative, weak positive, moderate positive, and strong positive expression. Histological grade was evaluated only in urothelial car-

cinoma cases. Pathological stage, muscular invasion, and lymphovascular invasion were evaluated only in cases with adequate histological representation.

2.8. Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics version 23. Categorical variables were summarized as frequencies and percentages. Associations between LEPR expression and categorical pathological variables were assessed using Pearson's chi-square test or Fisher's exact test, as appropriate. Fisher's exact test was used when expected cell counts were small. All tests were two-sided, and a *p* value below 0.05 was considered statistically significant. Normality testing was not performed because the main analyses were based on categorical variables. Because the study was retrospective and exploratory, *p* values were interpreted as evidence of association and not as proof of causality, mechanism, or independent prognostic value.

2.9. Ethics Approval

Ethical approval was obtained from the Ethics Committee of the National University of Sudan. Official authorization letters were issued to the participating institutions, and permission was granted to access archived FFPE tissue blocks. The study used archived diagnostic material and routinely collected clinicopathological data. All samples and data were anonymized before analysis, and the tissues were used only for research purposes. The study involved no direct patient contact, intervention, or identifiable patient information.

3. Results

3.1. Study Cohort and Clinicopathological Characteristics

A total of 153 histologically confirmed urinary bladder carcinoma cases were included. The cohort was predominantly male (120/153, 78.4%), with a male-to-female ratio of 3.6:1. Patient age ranged from 26 to 89 years. The largest age group was >59 years (77/153, 50.3%), followed by 40 - 59 years (68/153, 44.4%) and 18-39 years (8/153, 5.2%). Urothelial carcinoma was the most common histological subtype (136/153, 88.9%). Squamous cell carcinoma and adenocarcinoma accounted for 9/153 cases (5.9%) and 8/153 cases (5.2%), respectively. Histological grade was assessed only in urothelial carcinoma cases. Among the 136 urothelial carcinomas, 87 tumors (64.0%) were low grade and 49 (36.0%) were high grade.

Pathological pT category was assessable in 149 of 153 cases (97.4%). Among assessable cases, 52 tumors (34.9%) were Ta, 5 (3.4%) were Tis, 38 (25.5%) were T1, and 54 (36.2%) were T2 or higher. Four cases were not assessable for pathological pT category because of inadequate histological representation. Muscle invasion was present in 54/149 assessable cases (36.2%) and absent in 95/149 cases (63.8%). Lymphovascular invasion was identified in 56/149 cases (37.6%) and was absent in 93/149 cases (62.4%) (**Table 1; Figure 1**).

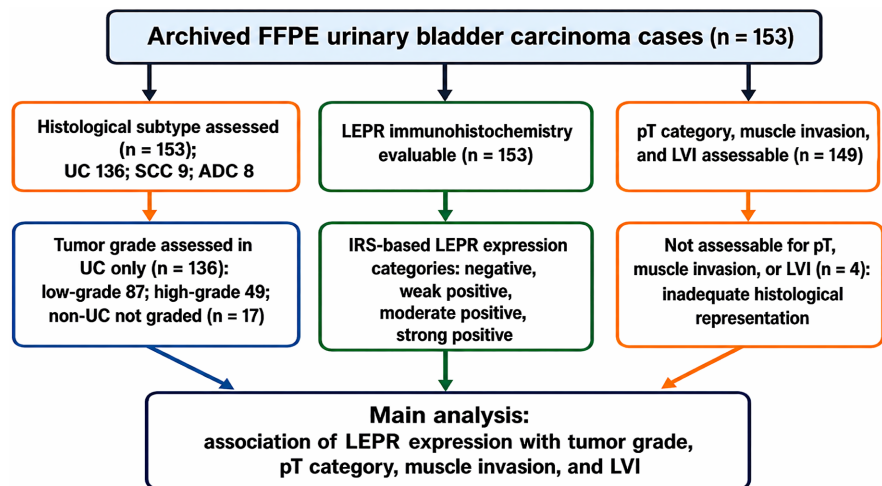


Figure 1. Study cohort and pathological analytical subsets. Flow diagram showing the 153 archived FFPE urinary bladder carcinoma cases included in the study. Histological subtype and LEPR immunohistochemistry were evaluated in all 153 cases. Tumor grade was assessed in 136 urothelial carcinoma cases. Pathological pT category, muscle invasion, and lymphovascular invasion were assessable in 149 cases; 4 cases were not assessable because of inadequate histological representation. Abbreviations: FFPE, formalin-fixed, paraffin-embedded; LEPR, leptin receptor; pT, pathological tumor category.

Table 1. Clinicopathological characteristics of the urinary bladder carcinoma cohort. Data are shown as n/N (%). Percentages were calculated using the denominator shown for each variable. The 95% confidence intervals were calculated using the Wilson method. Abbreviations: CI, confidence interval; pT, pathological tumor category. Tumor grade was assessed only in urothelial carcinoma. Pathological pT category, muscle invasion, and lymphovascular invasion were assessable in 149 cases.

Domain	Category	n/N (%)	95% CI
Sex	Male	120/153 (78.4)	71.3 - 84.2
Sex	Female	33/153 (21.6)	15.8 - 28.7
Age group, years	18 - 39	8/153 (5.2)	2.7 - 10.0
Age group, years	40 - 59	68/153 (44.4)	36.8 - 52.4
Age group, years	>59	77/153 (50.3)	42.5 - 58.1
Histological subtype	Urothelial carcinoma	136/153 (88.9)	82.9 - 92.9
Histological subtype	Squamous cell carcinoma	9/153 (5.9)	3.1 - 10.8
Histological subtype	Adenocarcinoma	8/153 (5.2)	2.7 - 10.0
Tumor grade*	Low grade	87/136 (64.0)	55.6 - 71.6
Tumor grade*	High grade	49/136 (36.0)	28.4 - 44.4
Pathological pT category [†]	Ta	52/149 (34.9)	27.7 - 42.8
Pathological pT category [†]	Tis	5/149 (3.4)	1.4 - 7.6
Pathological pT category [†]	T1	38/149 (25.5)	19.2 - 33.1
Pathological pT category [†]	T2 or higher	54/149 (36.2)	29.0 - 44.2

Continued

Muscle invasion [†]	Absent	95/149 (63.8)	55.8 - 71.0
Muscle invasion [†]	Present	54/149 (36.2)	29.0 - 44.2
Lymphovascular invasion [†]	Absent	93/149 (62.4)	54.4 - 69.8
Lymphovascular invasion [†]	Present	56/149 (37.6)	30.2 - 45.6

*Tumor grade was evaluated only in urothelial carcinoma cases; [†]Pathological pT category, muscle invasion, and lymphovascular invasion were assessable in 149 cases.

3.2. LEPR Immunohistochemical Expression and Pathological Indicators of Tumor Aggressiveness

LEPR immunohistochemical expression differed across pathological subgroups of urinary bladder carcinoma (**Table 2**). Representative staining showed LEPR expression in tumor cells, with predominantly cytoplasmic staining and focal membranous accentuation (**Figure 2**). The distribution of LEPR expression across pathological subgroups is shown in **Figure 3**. Among urothelial carcinomas with available grading data, moderate LEPR expression was more frequent in high-grade tumors than in low-grade tumors (14/49, 28.6% vs 9/87, 10.3%). Low-grade tumors more often showed negative expression (46/87, 52.9%), whereas high-grade tumors more often showed weak or moderate staining. The association between LEPR expression and histological grade was significant (Pearson χ^2 test, $p = 0.002^*$; Cramér's $V = 0.308$). LEPR expression also differed by pathological pT category. Moderate expression was observed in 7/52 Ta tumors (13.5%), 0/5 Tis tumors (0.0%), 9/38 T1 tumors (23.7%), and 19/54 T2-or-higher tumors (35.2%). Because of the small Tis subgroup, Fisher's exact test was used. The association between LEPR expression and pathological pT category was significant ($p = 0.002^*$; Cramér's $V = 0.254$).

A similar pattern was observed for muscle invasion. Moderate LEPR expression was more frequent in muscle-invasive bladder carcinoma than in non-muscle-invasive bladder carcinoma (19/54, 35.2% vs 18/95, 18.9%). Negative expression was more common in non-muscle-invasive tumors (52/95, 54.7%) than in muscle-invasive tumors (13/54, 24.1%). This association was significant (Pearson χ^2 test, $p = 0.001^*$; Cramér's $V = 0.299$). LEPR expression was also associated with lymphovascular invasion. Tumors with lymphovascular invasion more often showed weak or moderate LEPR staining, whereas tumors without lymphovascular invasion more often showed negative expression (51/93, 54.8%). The association between LEPR expression and lymphovascular invasion was significant (Pearson χ^2 test, $p = 0.001^*$; Cramér's $V = 0.297$).

Taken together, higher LEPR expression was more frequent in tumors with unfavorable pathological features, including higher tumor grade, higher pathological pT category, muscle invasion, and lymphovascular invasion. These findings support an association between LEPR expression and pathological indicators of tumor aggressiveness in this cohort.

Table 2. LEPR immunohistochemical expression according to pathological indicators of tumor aggressiveness. Data are shown as n (%). Percentages were calculated within each pathological subgroup. Abbreviations: LEPR, leptin receptor; LVI, lymphovascular invasion; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer; pT, pathological tumor category; χ^2 , chi-square. Pearson's χ^2 test was used for histological grade, muscle invasion, and lymphovascular invasion. Fisher's exact test was used for pathological pT category because of the small Tis subgroup. Cramér's V was used to estimate association strength. *p < 0.05.

Variable	Category	Negative n (%)	Weak positive n (%)	Moderate positive n (%)	Test	Cramér's V	p value
Histological grade	High grade (n = 49)	12 (24.5)	23 (46.9)	14 (28.6)	Pearson χ^2	0.308	0.002*
	Low grade (n = 87)	46 (52.9)	32 (36.8)	9 (10.3)	—	—	—
Pathological pT category	Ta (n = 52)	27 (51.9)	18 (34.6)	7 (13.5)	Fisher's exact	0.254	0.002*
	Tis (n = 5)	5 (100.0)	0 (0.0)	0 (0.0)	—	—	—
	T1 (n = 38)	13 (34.2)	16 (42.1)	9 (23.7)	—	—	—
	T2 or higher (n = 54)	13 (24.1)	22 (40.7)	19 (35.2)	—	—	—
Muscle invasion	MIBC (n = 54)	13 (24.1)	22 (40.7)	19 (35.2)	Pearson χ^2	0.299	0.001*
	NMIBC (n = 95)	52 (54.7)	25 (26.3)	18 (18.9)	—	—	—
Lymphovascular invasion	Present (n = 56)	14 (25.0)	28 (50.0)	14 (25.0)	Pearson χ^2	0.297	0.001*
	Absent (n = 93)	51 (54.8)	25 (26.9)	17 (18.3)	—	—	—

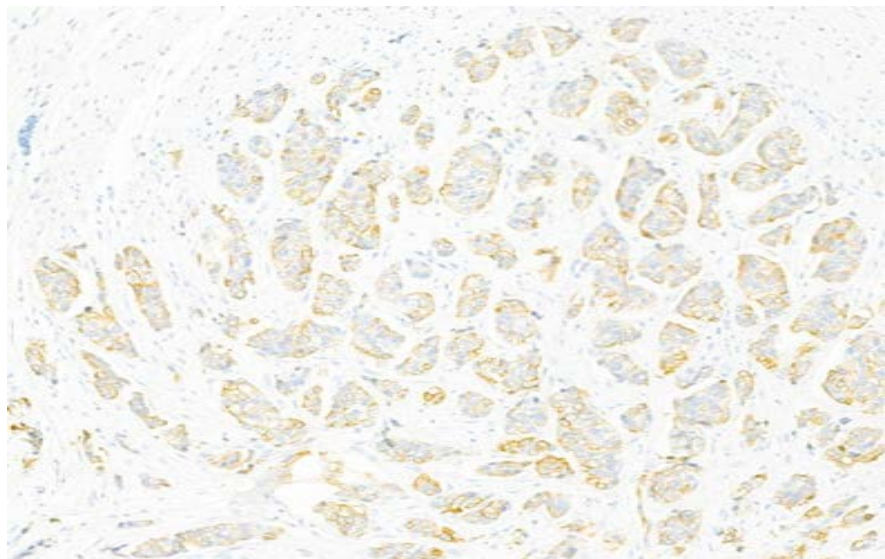


Figure 2. LEPR immunohistochemical expression in urinary bladder carcinoma. Representative immunohistochemical section showing LEPR expression in tumor cells. Staining is predominantly cytoplasmic, with focal membranous accentuation and weak-to-moderate intensity. Brown signal indicates 3,3'-diaminobenzidine chromogenic reactivity, with hematoxylin nuclear counterstain. Abbreviations: DAB, 3,3'-diaminobenzidine; IHC, immunohistochemistry; LEPR, leptin receptor.

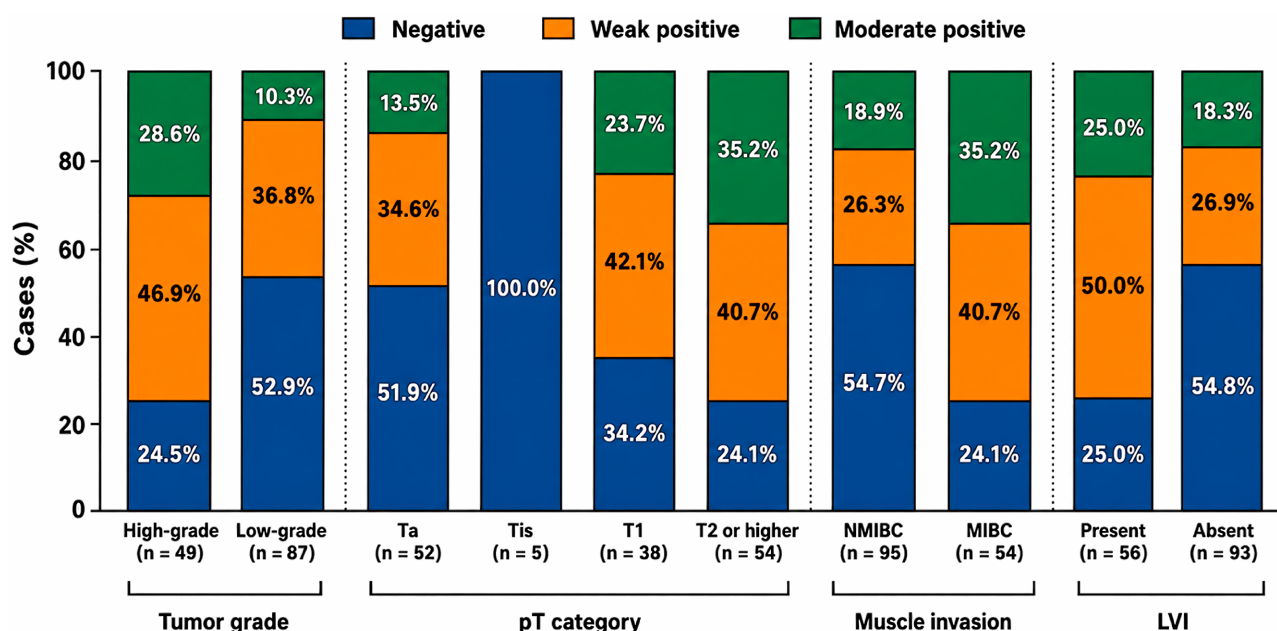


Figure 3. LEPR immunohistochemical expression across pathological subgroups. Stacked bar chart showing the distribution of negative, weak positive, and moderate positive LEPR staining across histological grade, pathological pT category, muscle invasion, and lymphovascular invasion status. Moderate LEPR expression was more frequent in high-grade tumors, T2-or-higher tumors, muscle-invasive tumors, and lymphovascular invasion-positive tumors. Abbreviations: LEPR, leptin receptor; LVI, lymphovascular invasion; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer; pT, pathological tumor category.

3.3. Unadjusted Association between LEPR Positivity and Adverse Pathological Features

To further investigate the direction and strength of association, LEPR expression was analyzed as a binary variable. Weak or moderate staining was classified as LEPR-positive, and negative staining was used as the reference category. These analyses were derived from the same contingency counts reported in **Table 2** and were interpreted as exploratory. LEPR positivity was more frequent in high-grade urothelial carcinoma than in low-grade urothelial carcinoma (37/49, 75.5% vs 41/87, 47.1%). In unadjusted analysis, high-grade tumors had higher odds of LEPR positivity than low-grade tumors (OR, 3.46; 95% CI, 1.59 - 7.51; $p = 0.002^*$). The unadjusted odds ratios for LEPR positivity across adverse pathological features are summarized in **Figure 4**.

A similar pattern was observed by pathological stage. LEPR positivity was more frequent in T2-or-higher tumors than in Ta, Tis, or T1 tumors combined (41/54, 75.9% vs 50/95, 52.6%). T2-or-higher tumors had higher odds of LEPR positivity than non-T2-or-higher tumors (OR, 2.84; 95% CI, 1.35 - 5.96; $p = 0.005^*$). LEPR positivity was also more common in muscle-invasive bladder carcinoma than in non-muscle-invasive bladder carcinoma (41/54, 75.9% vs 43/95, 45.3%). Muscle-invasive tumors had higher odds of LEPR positivity (OR, 3.81; 95% CI, 1.81 - 8.02; $p < 0.001^*$). Tumors with lymphovascular invasion showed a similar pattern. LEPR positivity was detected in 42/56 LVI-positive tumors (75.0%) compared with 42/93 LVI-negative tumors (45.2%). LVI-positive tumors had higher odds of

LEPR positivity than LVI-negative tumors (OR, 3.64; 95% CI, 1.76 - 7.56; $p < 0.001^*$). The unadjusted association between LEPR positivity and adverse pathological features is presented in **Table 3**.

These unadjusted estimates support the main categorical findings and show that LEPR positivity was consistently enriched in tumors with adverse pathological features. Because the study was retrospective and did not include survival data or multivariable modeling, these estimates should be interpreted as evidence of association, not as proof of independent prognostic value.

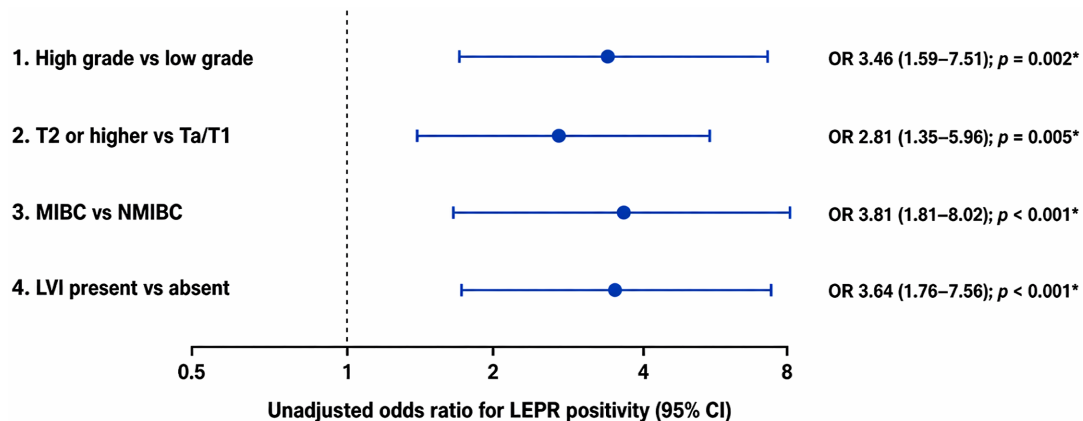


Figure 4. Unadjusted odds ratios for LEPR positivity across adverse pathological features. Forest plot showing unadjusted odds ratios and 95% confidence intervals for LEPR positivity across adverse pathological features. LEPR positivity was defined as weak positive or moderate positive staining; negative staining was used as the reference category. Abbreviations: CI, confidence interval; LEPR, leptin receptor; LVI, lymphovascular invasion; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer; OR, odds ratio; pT, pathological tumor category.

Table 3. Unadjusted association between LEPR positivity and adverse pathological features. LEPR positivity was defined as weak positive or moderate positive staining. Negative staining was used as the reference category. Odds ratios were calculated from 2×2 contingency tables. Abbreviations: CI, confidence interval; LEPR, leptin receptor; LVI, lymphovascular invasion; MIBC, muscle-invasive bladder cancer; NMIBC, non-muscle-invasive bladder cancer; OR, odds ratio; pT, pathological tumor category. $^*p < 0.05$.

Comparison	LEPR-positive in adverse group n/N (%)	LEPR-positive in reference group n/N (%)	OR (95% CI)	p value
High grade vs low grade	37/49 (75.5)	41/87 (47.1)	3.46 (1.59 - 7.51)	0.002*
T2 or higher vs Ta/Tis/T1	41/54 (75.9)	50/95 (52.6)	2.84 (1.35 - 5.96)	0.005*
MIBC vs NMIBC	41/54 (75.9)	43/95 (45.3)	3.81 (1.81 - 8.02)	<0.001*
LVI present vs absent	42/56 (75.0)	42/93 (45.2)	3.64 (1.76 - 7.56)	<0.001*

4. Discussion

This study evaluated LEPR immunohistochemical expression in 153 archived urinary bladder carcinoma tissues from Sudanese patients. LEPR expression showed a consistent association with pathological indicators of tumor aggressiveness. Moderate expression was more frequent in high-grade urothelial carcinoma, T2-or-higher tumors, muscle-invasive tumors, and tumors with lymphovascular in-

vasion. In secondary binary analyses, LEPR positivity was also enriched in the same adverse pathological groups. These findings suggest that LEPR expression is linked to a more aggressive pathological phenotype in this cohort. They do not prove independent prognostic value, because survival and treatment-response data were not available.

A notable finding was the consistency of the association across several pathological endpoints. Tumor grade, pathological stage, muscularis propria invasion, and lymphovascular invasion are established markers used in bladder cancer risk assessment and clinical decision-making [1] [4]-[6]. The association between LEPR expression and these features therefore has biological and pathological relevance. The relationship with muscular invasion is especially important, because the transition from non-muscle-invasive to muscle-invasive disease marks a major change in clinical behavior and treatment strategy. The association with lymphovascular invasion is also important, because LVI reflects a pathway for tumor dissemination and has been linked to poorer outcomes in bladder cancer cohorts and meta-analyses [16]. In addition, it has been shown that key tumor-related factors, including tumor burden and stages may allow patients to be grouped into lower- and higher-risk categories for clinical follow-up and management [17].

The present findings are broadly consistent with earlier tissue-based studies of leptin-related signaling in urothelial tumors. Khabaz *et al.* reported that leptin expression in urinary bladder carcinoma was associated with advanced stage, muscularis propria invasion, vascular invasion, nodal involvement, distant metastasis, and poorer survival [9]. Kashiwagi *et al.* also found that adipocytokines and their receptors were expressed in bladder cancer and that leptin-related expression had prognostic relevance, particularly in muscle-invasive disease [10]. In upper tract urothelial carcinoma, Lee *et al.* reported that LEPR overexpression was associated with poorer recurrence-free and cancer-specific survival [11]. Taken together, these studies support the view that leptin-LEPR signaling may be linked to aggressive urothelial tumor biology.

These observations are biologically plausible given the established role of leptin signaling in cancer-related pathways. LEPR activation can engage JAK2/STAT3, PI3K/Akt, and MAPK signaling pathways, which are involved in cell survival, proliferation, inflammation, angiogenesis, invasion, and treatment resistance [18]. Leptin biology is also closely connected to immune regulation and chronic inflammation, both of which may shape the tumor microenvironment [18] [19]. In bladder cancer, these mechanisms remain incompletely defined. The current study did not assess pathway activation, serum leptin levels, obesity, metabolic status, or tumor immune composition. Therefore, the observed IHC associations should be interpreted as tissue-level evidence of association, not as proof of a causal mechanism.

This cohort contributes data from a population that is poorly represented in biomarker studies of bladder carcinoma. This is relevant because genetic background, environmental exposure, chronic inflammation, infection-related factors,

and patterns of presentation may differ across regions. The inclusion of all histological subtypes reflects real diagnostic practice. However, grade-based analysis was restricted to urothelial carcinoma, because WHO/ISUP grading is not routinely applied in the same way to squamous cell carcinoma or adenocarcinoma. This distinction should be retained in the manuscript to avoid overgeneralization.

Several limitations should be considered. First, the retrospective cross-sectional design limits causal inference and may introduce selection bias related to tissue availability and preservation. Second, survival, recurrence, progression, and treatment-response data were unavailable. For this reason, tumor grade, stage, muscular invasion, and lymphovascular invasion were used as pathological indicators of aggressiveness, not as direct clinical endpoints. Third, the sample size was moderate, and some subgroups were small, especially carcinoma in situ. Fourth, IHC scoring is semi-quantitative and may be affected by tissue fixation, antigen preservation, antibody performance, tumor heterogeneity, and observer variation. The use of a defined IRS system, blinded scoring, and reproducibility assessment reduces this concern but does not eliminate it. Fifth, the absence of matched normal urothelium limits interpretation of tumor-specific overexpression. Finally, the analysis did not include multivariable modeling, so the independent contribution of LEPR expression beyond established pathological factors remains uncertain.

Larger prospective studies should evaluate LEPR expression in well-characterized bladder cancer cohorts with survival, recurrence, progression, and treatment-response outcomes. Future work should also include matched normal tissue, standardized digital image analysis, obesity and metabolic data, and pathway-level validation. If confirmed, LEPR may help identify biologically aggressive tumors and may support future studies of adipokine-mediated signaling in bladder cancer. LEPR is better considered as a candidate tissue biomarker associated with adverse pathological features rather than validated prognostic marker for clinical use.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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