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Clinical Implications of Hypoxia-Inducible Factor-1α Expression in Bladder Cancer

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Abstract: Aim: Hypoxia-inducible factor-1α (HIF-1α) is a central regulator of tumor biology, enabling bladder cancer cells to adapt under oxygen-deprived conditions. This study intended to assess the clinical significance of HIF-1α expression in bladder cancer (BC) and its correlation with key pathological parameters. **Methods:** Blood was obtained from 110 cases diagnosed with BC and from 110 age- and sex-matched healthy volunteers who served as controls. HIF-1α mRNA expression was quantified using real-time PCR. **Results:** Histological evaluation revealed that the majority of cases (95.5%) were urothelial carcinoma, while 4.5% were squamous cell carcinoma (SCC). Elevated HIF-1α transcript levels were considerably linked to the SCC subtype ($p = 0.04$). ROC curve analysis demonstrated that HIF-1α could reliably distinguish BC patients from controls (AUC = 0.998, $p < 0.001$) at a threshold value of 1.5. **Conclusions:** The data underscore the importance of HIF-1α overexpression in BC progression. Its increased expression was linked to unfavorable clinicopathological traits, highlighting its potential as a prognostic indicator. Owing to its involvement in angiogenesis, metabolic alterations, and treatment resistance, HIF-1α may represent an attractive therapeutic target. Nevertheless, validation in large, prospective cohorts is required to clarify its prognostic and predictive value and to establish the clinical utility of HIF-1α–targeted interventions in BC management.

keywords: Bladder cancer, HIF-1α gene expression, qpcr.

1.Introduction

Bladder cancer (BC) is among the most common urological malignancies, ranking seventh in incidence among men and approximately tenth overall when both sexes are considered. According to global cancer statistics for 2020, an estimated 573,000 new cases were identified worldwide, with over 212,000 deaths attributed to the disease. From a clinical perspective, BC is generally classified into two principal categories: non–muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC) (1).

NMIBC includes carcinoma in situ, papillary noninvasive tumors, and tumors invading only the lamina propria, accounting for nearly 70% of cases at diagnosis. In contrast, about 30% of patients present with MIBC, which invades the detrusor muscle and carries a higher risk of lymphatic and distant metastasis (2).

MIBC represents the most aggressive subtype, with a 5-year survival rate of approximately 60% in localized disease but less than 10% in advanced metastatic cases (3). At present, the recommended management approach involves administering neoadjuvant chemotherapy (NAC) prior to radical cystectomy. Nevertheless, a meaningful pathological response is observed in only about one-quarter to one-half of treated patients. (4). Consequently, identifying reliable biomarkers to predict treatment outcomes is of critical importance, as non-responders face unnecessary toxicity and potential delays in curative surgery.

Angiogenesis is a hallmark of tumor progression, enabling cancer cells to meet increased metabolic demands and facilitating metastatic spread. These processes are strongly influenced by cellular adaptation to hypoxia and metabolic reprogramming, both of which are central to cancer biology and treatment resistance (5). Tumor hypoxia has been strongly associated with aggressive biological behavior, including enhanced invasiveness, distant spread, unfavorable prognosis, and reduced responsiveness to chemotherapy, radiotherapy, and immunotherapy. Among the angiogenic factors, vascular endothelial growth factor (VEGF) is particularly prominent, and its induction is largely controlled by HIF-1α. This transcriptional regulator is a key mediator of cellular adaptation under oxygen-deprived conditions. (7).

HIF-1 is a transcriptional complex consisting of two components: HIF-1α and HIF-1β. Under normoxic conditions, HIF-1α is rapidly degraded via the ubiquitin–proteasome system, a process that requires recognition by the von Hippel–Lindau (VHL) tumor suppressor protein. When oxygen availability is limited, this degradation pathway is inhibited, allowing HIF-1α to accumulate. The stabilized subunit then associates with HIF-1β, and the active dimer binds to hypoxia-responsive elements in the promoters of target genes (8). As a consequence, numerous genes responsive to low oxygen are transcriptionally activated, such as VEGF, glucose transporters, erythropoietin, and key enzymes of glycolysis. Many of these targets are frequently overexpressed in malignancies and contribute to more aggressive phenotypes. Notably, increased VEGF expression has been correlated with disease progression and an unfavorable relapse-free survival rate in patients with NMIBC (9).

Building on this rationale, our study was designed to examine the clinical relevance of HIF-1α expression in BC and explore its connection with major clinicopathological structures, with a specific motivation on its value as a prognostic indicator and likely therapeutic target.

2. Materials and methods

Study population

This investigation included 110 individuals diagnosed with bladder cancer (BC) and treated at the Urology and Nephrology Center, Mansoura, Egypt. Ethical approval for the study (MS.20.06.1166) was obtained from the Faculty of Medicine, Mansoura University, and informed consent was secured from every participant prior to participation. The control group comprised of 110 healthy volunteers with matched age and sex. Relevant clinical, and disease characteristics of study subjects were retrieved from registered data archives.

Methods

Three milliliters of venous blood were withdrawn on EDTA tube for real-time PCR to detection of HIF-1α expression level.GENEzol™ TriRNA Pure Kit was for purification of high-quality RNA from the blood samples. The isolated RNA was reverse transcribed into cDNA using the COSMO cDNA PLUS synthesis kit (WF 10205006, Lot: 04fp62231) on a Veriti Real-Time PCR system (Applied Biosystems, Singapore; S/N 2990226743). Quantitative gene expression was subsequently assessed with the SYBR® Green qPCR assay employing the Willowfort HERAPLUS SYBR Green kit. The PCR amplification was done using Stepone Real Time PCR instrument. Each PCR reaction consisted of: 10µl Master Mix (2x), 1µl of both forward (5′- GTCTGAGGGGACAGGAGGAT -3′) and reverse primers (5′- GCACCAAGCAGGTCATAGGT-3′) (200nM), 250ng of DNA template and 20µl nuclease free water. The cycling profile involved an initial denaturation step at 95 °C for 10 minutes, then 40 repeated cycles of denaturation at 95 °C for 15 seconds, with annealing and extension carried out at 60 °C for 1 minute.

Statistical analysis

Continuous variables with parametric distribution were expressed as mean ± SD, whereas categorical variables were summarized as numbers and percentages. Comparisons between study groups for normally distributed quantitative variables were carried out using the Student's t-test. Associations between categorical variables were assessed by the Chi-

square test when applicable. Diagnostic performance of quantitative measures was assessed through receiver operating characteristic (ROC) curve analysis, which determines sensitivity and specificity across potential thresholds. The optimal cut-off value was defined as the point yielding the maximum area under the curve (AUC). An AUC greater than 0.9 was interpreted as excellent accuracy, 0.7–0.9 as moderate, 0.5–0.7 as low, and 0.5 as no better than chance. Statistical significance was accepted at a p-value < 0.05.

3. Results and Discussion

Results

The age of the BC patients was 66.79 ± 7.89 years, ranged from 48.0 to 85.0 years, with (87.27%) males and (12.27%) females. The higher percentage of patients (48%) was between 66 – 75 years, with no significant difference with the controls regarding age and sex (p=0.408 and p=0.248, respectively). **Figure 1** shows that (73.64%) of patients presented with hematuria, (14.55%) with loin pain, (5.45%) with irritative LUTS, (3.64%) with irritative LUTS and (2.72%) with necroturia.

According to the histological type of the tumor, (95.45%) of patients presented with Urothelial carcinoma and (4.55%) with SCC. Regarding the tumor grade, (73.64%) of patients presented with grade 1, (3.64%) with grade 2 and (5.45%) with grade 3. The tumor stage was determined by the TNM system, which classified the patients as the following [T: (T1: 11.81%, T2a: 15.45%, T2b: 15.45, T3a: 36.39, T3b:17.27 and T4: 3.63), N: (N0: 73.63%, N1: 9.1% and N2: 17.27%) and M: (M0: 90.9% and M1:9.1%], as displayed in **(Table 1).**

Table 2 shown that higher H1F1-α expression was associated with SCC histological type (p=0.04), **(Figures 3).** No other significant differences were recorded. ROC curve analysis showed that HIF-1α were significant predictors to discriminate between the healthy controls and the BC with excellent AUC (0.998) at cut-off point (1.5), **(Table 3).**

Discussion:

The present analysis demonstrated a significant correlation between HIF-1α expression and aggressive pathological characteristics of bladder cancer, including increased grade and stage. This supports the hypothesis that HIF-1α functions as an important driver of tumor adaptation under hypoxic stress contributes to the development and progression of bladder malignancy.

Hypoxia is a well-recognized hallmark of solid tumors, and the stabilization of HIF-1α under low oxygen conditions activates a transcriptional program that promotes angiogenesis, metabolic reprogramming, and resistance to apoptosis, ultimately facilitating tumor progression and therapeutic resistance (10).

Our findings align with earlier studies that emphasized the prognostic relevance of HIF-1α in urothelial carcinoma. For instance, Theodoropoulos and colleagues established that elevated HIF-1α expression correlated with higher microvessel density and poorer disease-specific survival among patients with bladder transitional cell carcinoma. (11). Similarly, Wu et al. informed that HIF-1α overexpression related to muscle invasion and shorter recurrence-free survival, suggesting its role as an independent prognostic marker (12). More recently, Fan et al. emphasized the clinical impact of hypoxia-driven molecular pathways in bladder cancer progression, which further aligns with our findings. Collectively, these observations reinforce the relevance of HIF-1α as a biomarker of tumor aggressiveness. (1).

The biological basis for these clinical associations is complex. By regulating the expression of VEGF, HIF-1α facilitates angiogenesis, a process essential for tumor growth and dissemination. (13, 14). In addition, HIF-1α enhances the expression of glucose transporters such as GLUT-1, together with several glycolytic enzymes, thereby driving cancer cells toward anaerobic glycolysis—the so-called Warburg effect. This metabolic shift allows cells to maintain energy generation even under hypoxic stress. Beyond supporting survival, it also contributes to the development of an acidic tumor microenvironment, which favors invasion, metastatic dissemination, and evasion of immune surveillance. In addition, both hypoxia and HIF-1α activation have been linked to the induction of epithelial–

mesenchymal transition (EMT), a mechanism that may account for the observed association between HIF-1α expression and muscle-invasive disease in our cohort (17).

A key clinical relevance of our results lies in their potential to predict treatment outcomes. For patients with muscle-invasive bladder cancer, NAC followed by radical cystectomy remains the recommended therapeutic approach; nevertheless, only a proportion of cases demonstrate a substantial pathological response. Evidence from previous studies indicates that tumor hypoxia and elevated HIF-1α expression are linked to reduced responsiveness to cisplatin-based chemotherapy (18).

For instance, Hoskin et al. demonstrated that tumors with hypoxia markers exhibited lower chemosensitivity, while Choudhury et al. suggested that HIF-1α overexpression could predict radiotherapy resistance. Our findings, therefore, may have potential predictive significance, suggesting that patients with high HIF-1α expression could be stratified for alternative or intensified treatment regimens, including hypoxia-targeted therapies. (19, 20).

Therapeutic strategies aimed at inhibiting HIF-1α or its downstream signaling cascades are increasingly recognized as promising in cancer management. Experimental models have demonstrated that pharmacological blockade of HIF-1α can reduce angiogenesis, improve tumor radiosensitivity, and re-establish chemosensitivity. For example, agents such as PX-478 and acriflavine have been explored as HIF-1α inhibitors in experimental models, with encouraging results (21). In the context of bladder cancer, combining HIF-1α inhibition with standard therapies may improve outcomes by overcoming hypoxia-mediated resistance. While our study did not directly evaluate therapeutic interventions, the observed correlation between HIF-1α expression and tumor aggressiveness underscores the rationale for exploring HIF-1α as a therapeutic target in future clinical trials. Targeting HIF-1α or its downstream pathways has emerged as a promising therapeutic strategy in oncology. Preclinical studies have shown that pharmacological inhibition of HIF-1α can suppress angiogenesis, enhance radiosensitivity, and restore chemosensitivity (21). Although this study has notable strengths, such as the systematic assessment of HIF-1α expression and its relationship with clinicopathological variables, certain limitations must be considered. The relatively small cohort size is the first limitation, which may restrict both the statistical robustness and the external validity of the results. Second, immunohistochemistry, although widely used, is a semi-quantitative method and may be influenced by technical variability and the choice of cut-off values. Third, this study was cross-sectional in design, precluding a direct assessment of the impact of HIF-1α on treatment outcomes or survival. Validation of our results will require larger-scale studies with consistent assessment protocols and long-term follow-up to define the standalone prognostic and predictive role of HIF-1α.

Future investigations should focus on validating HIF-1α as both a prognostic and predictive biomarker in larger, multicenter cohorts with long-term clinical follow-up. Integrating HIF-1α assessment into biomarker panels that include angiogenic and metabolic markers such as VEGF, GLUT-1, and CAIX may improve risk stratification and treatment decision-making in bladder cancer. Moreover, the therapeutic potential of HIF-1α inhibition warrants further investigation. Preclinical studies have confirmed that pharmacological inhibitors of HIF-1α, including PX-478 and acriflavine, can suppress angiogenesis and enhance sensitivity to chemotherapy and radiotherapy. Combining these agents with standard-of-care regimens such as cisplatin-based chemotherapy or immune checkpoint inhibitors could overcome hypoxia-induced treatment resistance. In addition, the development of non-invasive methods for detecting tumor hypoxia, such as hypoxia-specific PET tracers or circulating biomarkers, may facilitate real-time monitoring of tumor biology and guide personalized therapeutic strategies. Ultimately, translating these insights into clinical practice will require well-designed prospective trials to evaluate the safety, efficacy, and feasibility of HIF-1α–targeted interventions in bladder cancer patients.

In conclusion, These findings support the pivotal role of HIF-1α in bladder tumor

progression, where its overexpression correlates with poor pathological features and mechanisms such as angiogenesis, altered metabolism, and therapy resistance. These results highlight the promise of HIF-1α as both a prognostic indicator and a potential therapeutic target in bladder cancer. To establish its role in guiding clinical management and to evaluate the benefits of HIF-1α–directed therapies, well-designed large-scale prospective studies and interventional trials are still required.

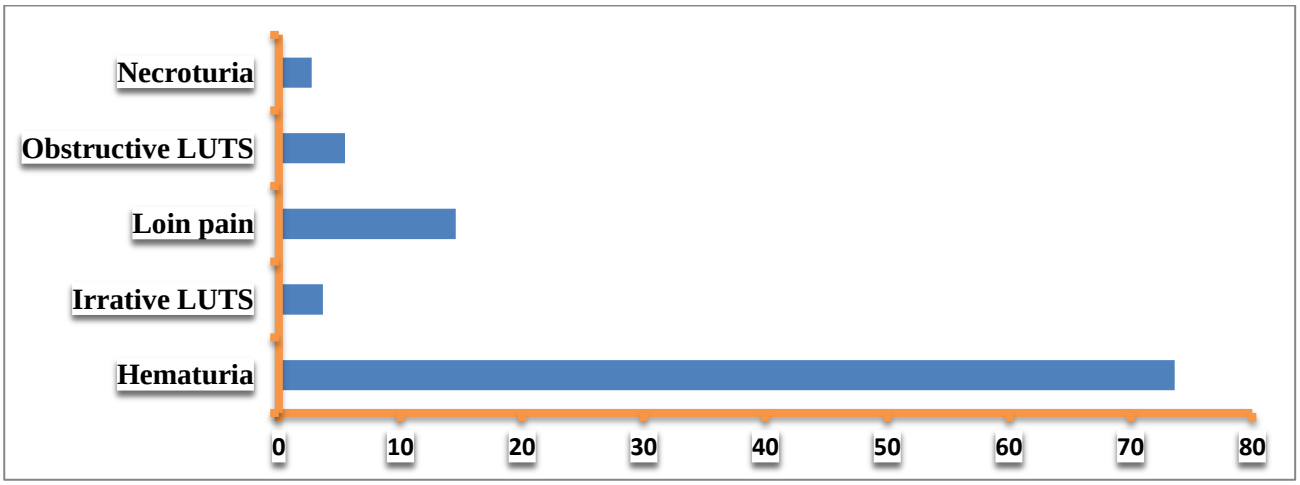

Figure (1): Clinical presentation of the studied patients.

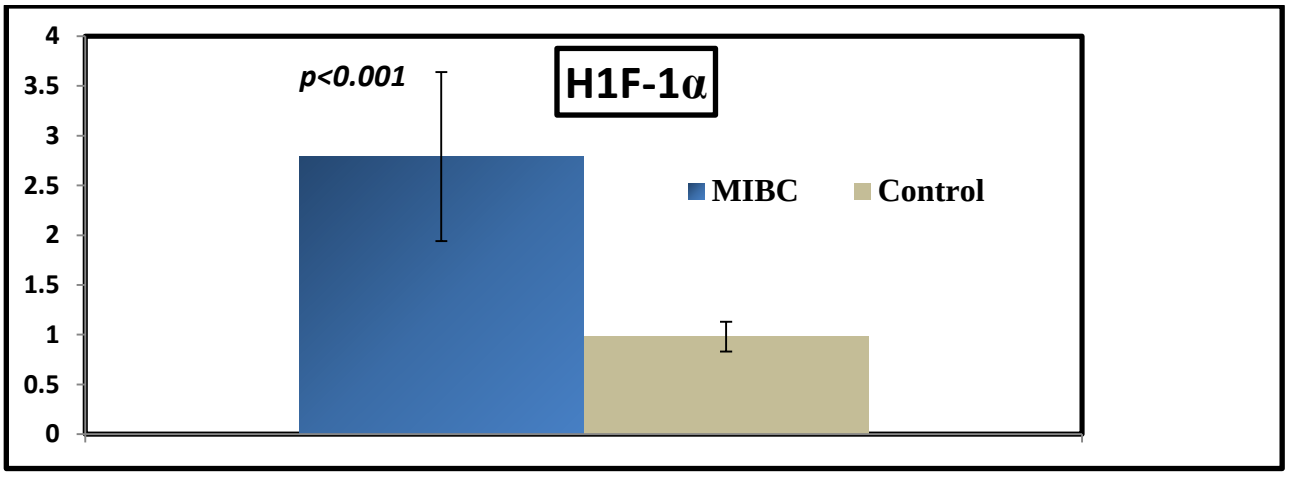

Figure (2): Comparison of H1F-1α expression between BC group and control group**.**

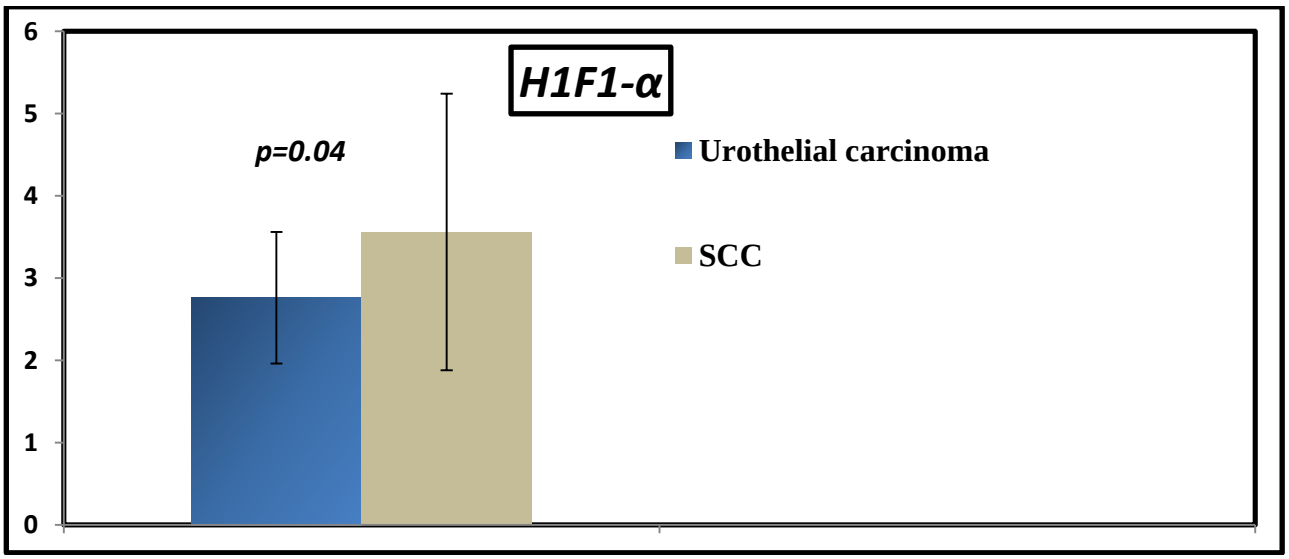

Figure (3): Comparison of H1F1-α expression regarding histological type.

Table (1): Clinopathological characteristics of the studied patients.

	BC		
	n		%
Histological type			
Urothelial carcinoma	105		95.45
SCC	5		4.55
Tumor grade	81		73.64
G1	4		3.64
G2	16		14.55
G3	6		5.45
Tumor stage	3		2.72
T stage			
T1	13		11.81
T2 (n=34)	**T2a**	17	15.45
	T2b	17	15.45
T3 (n=59)	**T3a**	40	36.39
	T3b	19	17.27
T4	4		3.63
N stage			
N0	81		73.63
N1	10		9.1
N2	19		17.27
M stage			
M0	100		90.9
M1	10		9.1

SCC: squamous cell carcinoma.

Table (2): Association of H1F1-α expression with demographic data and clinopathological characteristics

	H1F1-αexpression	**Test of significance**	***p-value***
Sex			
Males	2.78 ± 0.82	t=0.16	*p=0.87*
Females	2.82 ± 1.08		
Tumor grade			
G1	2.78 ± 0.87	F=0.06	*p=0.93*
G2	2.76 ± 0.57		
G3	2.9 ± 1.0		
T stage			
T1+T2	2.65 ± 0.78	t=1.23	*p=0.21*
T3+T4	2.85 ± 0.88		
N stage			
N0	2.74 ± 0.12	t=1.09	*p=0.27*
N1+N2	2.94 ± 0.84		
M stage			
Mo	2.78 ± 0.82	t=0.21	*p=0.83*
M1	2.84 ± 1.22		
Histological type			
Urothelial carcinoma	2.76 ± 0.80	t=2.05	***p=0.04****
SCC	3.56 ± 1.68		

**: significant (p<0.05), SCC: squamous cell carcinoma.*

Table (3): ROC analysis of the *H1F1-α* expression to discriminate between the BC and controls.

	HIF-1α
p-value	**<0.001****
AUC	**0.98**
Cut-off	**>1.5**
Sensitivity	**100%**
Specificity	**100%**
Accuracy	**100%**
PPV	**100%**
NPV	**100%**

PPV: positive predictive value, NPV: negative predictive value, **: highly significant (p<0.001).

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