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ORIGINAL ARTICLE

The Significance of Prolyl 4-Hydroxilase Beta Polypeptide Expression in Urothelial Carcinoma

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ABSTRACT

Background: Prolyl-4-hydroxylase subunit beta (P4HB) is an endoplasmic reticulum chaperone implicated in tumor progression, but its clinicopathological significance in urothelial carcinoma remains unclear. This research aimed to evaluate the expression of P4HB in urothelial carcinoma of the urinary bladder and examine its association with key clinicopathological features.

Methods: A retrospective study included 94 cases of urothelial carcinoma. Formalin-fixed, paraffin-embedded tumor samples were examined histologically and immunohistochemically for P4HB expression. Associations between P4HB expression and age, sex, tumor size, grade, stage, muscle invasion, and concomitant carcinoma in situ (CIS) were statistically analyzed.

Results: High P4HB expression was found in 60.3% of tumors >3 cm, compared to 15.4% of those ≤3 Cm ($p<0.001$). Among high-grade tumors, 93.3% showed high P4HB expression, while only 22.4% of low-grade tumors were P4HB-high ($p<0.001$). Muscle-invasive tumors demonstrated high P4HB expression in 77.4% of cases, in contrast to 9.8% in non-muscle-invasive tumors ($p<0.001$). No significant relationship was observed between P4HB expression and age ($p=0.504$) or sex ($p=0.628$). Concomitant CIS was present in 58.3% of cases with high P4HB expression, but this difference did not reach statistical significance ($p=0.235$).

Conclusions: Increased P4HB expression is significantly linked with larger tumor size, higher grade, advanced stage, and muscle invasion in urothelial carcinoma. P4HB immunostaining may be helpful as a marker for aggressive disease and could support early detection and prognostic assessment in bladder cancer.

Keywords: Prolyl 4-Hydroxilase Beta Polypeptide, Expression, Urothelial Carcinoma.

INTRODUCTION

Bladder cancer is recognized as the tenth most prevalent malignancy globally, as it remains a significant contributor to cancer-related mortality [1]. Men are affected about three to four times more often than women, a pattern seen across many countries [2]. In Egypt, bladder cancer stands out as the fourth most common tumor and is one of the significant causes of cancer deaths. Here, chronic infection with *Schistosoma haematobium* and tobacco use are key risk factors [3].

Bladder carcinomas are classified into urothelial and non-urothelial types. The urothelial category includes subtypes like those with squamous features, sarcomatoid changes, micropapillary and plasmacytoid variants, while non-urothelial tumors involve squamous cell carcinoma, adenocarcinoma, and neuroendocrine tumors [4]. Prognosis is strongly influenced by how deeply the tumor

invades the bladder wall as well as the tumor grade. Urothelial cancers are generally divided into non-muscle-invasive forms and muscle-invasive forms. While most cases are diagnosed at the non-muscle-invasive stage, about half will recur, and a considerable portion can progress to muscle invasion, especially if the tumor is of high grade [5].

The endoplasmic reticulum (ER) is critical for protein folding and calcium regulation at the cellular level. Stressful conditions such as low oxygen can disrupt ER function, causing a buildup of abnormal proteins [6]. In response, cells activate the unfolded protein response (UPR) to restore ER balance. Among the key components of this response are the protein-disulfide isomerases (PDIs), with prolyl-4-hydroxylase subunit beta (P4HB) being an important member [7]. P4HB acts as a molecular chaperone, helping to prevent protein aggregation, and assists in maintaining ER stability by modulating disulfide bonds.

Recent research has shown that P4HB is upregulated among various cancers, including bladder cancer, and plays a role in tumor progression, likely by supporting tumor cells under stressful conditions like hypoxia [8]. Experimental studies have also suggested that reducing P4HB levels in bladder cancer cells leads to decreased invasion and increased sensitivity to chemotherapy [9]. Moreover, P4HB is thought to be involved in key processes such as epithelial-mesenchymal transition (EMT) and cell migration, which are critical for cancer spread.

The classification of tumor staging included: primary tumor (pT), ranging from pTa (noninvasive papillary) to pT4b (extension to the abdominal wall); regional lymph nodes (pN), from pN0 (no metastasis) to pN3 (common iliac node involvement); and (pM), which comprised M0 (no distant metastasis), M1a (metastasis to nonregional distant metastasis lymph nodes), and M1b (metastasis to distant sites) [10,11]. Stage grouping was assigned based on the above criteria. For analysis, tumors were further stratified into non-muscle-invasive (Tis, Ta, T1) and muscle-invasive (T2–T4) categories [12].

Despite these growing insights, the clinical utility of P4HB as a biomarker in urothelial carcinoma remains to be fully elucidated. Most existing studies have focused on the molecular functions of P4HB or its role in other tumor types, leaving a significant knowledge gap regarding its expression patterns in bladder cancer and their relationship with established pathological parameters. Understanding whether P4HB correlates with features of tumor aggressiveness, such as grade, stage, or muscle invasion, may offer valuable prognostic information and potentially guide future management strategies.

Despite these findings, there is still limited data on how P4HB expression relates to clinicopathological factors and disease outcomes in urothelial carcinoma, especially in our region. Addressing this gap may help understand its potential as a diagnostic or prognostic marker. Therefore, this research aimed to evaluate the expression of P4HB in urothelial carcinoma and analyze its association with available clinicopathological parameters.

METHODS

We conducted this retrospective study at the Pathology Department, Faculty of Medicine, Zagazig University. The research included 94 formalin-fixed, paraffin-embedded (FFPE) tissue blocks from cases d iagnosed with urothelial carcinoma of the urinary bladder. These specimens were collected over a period from October 2022 to January 2025. All samples were obtained via transurethral resection of the bladder (TURB) after receiving approval number (10665/2/4-2023) from the local ethical committee and institutional review board (IRB) of the Faculty of Medicine, Zagazig University. The research was conducted under the World Medical Association's Code of Ethics (Helsinki Declaration) for human research.

Inclusion criteria required that cases be histologically confirmed urothelial carcinoma of the urinary bladder with muscularis propria present in the examined sections, availability of

complete clinical and histopathological records, and no history of chemotherapy before surgical specimen collection.

Exclusion criteria were cases if they were non-urothelial types of bladder carcinoma or if the biopsies provided insufficient tissue material for evaluation.

Clinical information, including age, sex, tumor size, and tumor grade at the time of diagnosis, was retrieved from the patients' medical records.

Histopathological Assessment

Sections measuring 3–4 micrometers in thickness were prepared from formalin-fixed, paraffin-embedded tissue blocks and subsequently stained with hematoxylin and eosin to allow for conventional histopathological examination under the microscope. Tumor grading was determined using the 2022 World Health Organization (WHO) criteria for urothelial carcinoma, classifying each case as either low or high [10]. Pathologic staging was conducted following the 8th edition of the American Joint Committee on Cancer (AJCC) TNM staging system, evaluating the extent of primary tumor, regional lymph node involvement, and presence of distant metastasis.

The classification of tumor staging included: **primary tumor** (pT), ranging from pTa (noninvasive papillary) to pT4b (extension to the abdominal wall); **regional lymph nodes** (pN), from pN0 (no metastasis) to pN3 (common iliac node involvement); and (pM), which comprised M0 (no distant metastasis), M1a (metastasis to nonregional **distant metastasis** lymph nodes), and M1b (metastasis to distant sites) [11].

Stage grouping was assigned based on the above criteria. For analysis, tumors were further stratified into non-muscle-invasive (Tis, Ta, T1) and muscle-invasive (T2–T4) categories [12].

Immunohistochemical Analysis

Immunohistochemical (IHC) staining for P4HB was done using the streptavidin-biotin immunoperoxidase technique. The primary antibody was a rabbit monoclonal anti-P4HB (ABclonal, ARC2398 clone), at a recommended dilution of 1:200.

Immunohistochemistry (IHC) procedures were conducted according to established protocols [13]: Sections were cut at 3–5 μm thickness and mounted onto poly-L-lysine-coated slides. Deparaffinization was carried out in xylene and rehydrated through a series of graded alcohols. Antigen retrieval was achieved using Dako target retrieval solution (EDTA, pH 6.0) in a microwave oven. Endogenous peroxidase activity was quenched with 3% hydrogen peroxide. After rinsing in phosphate-buffered saline (PBS), the primary antibody was applied, and the slides were incubated overnight at 2–8°C in a humidified chamber. Immunodetection was performed using biotinylated secondary antibodies, streptavidin–horseradish peroxidase (HRP), and diaminobenzidine (DAB) as the chromogen. Slides underwent counterstaining with Mayer's hematoxylin, then were dehydrated, cleared, and coverslipped. Quality assurance included using a human placenta as a positive control, while negative controls were established by omitting the primary antibody during immunostaining.

Immunostaining Evaluation

P4HB immunoreactivity was evaluated in both the cytoplasmic and membranous compartments, with expression scored semiquantitatively by two independent pathologists blinded to clinical data, using the following criteria: staining intensity (1: none, 2: weak, 3: moderate, 4: strong) and proportion of positive cells (1: ≥1%–<25%, 2: ≥25%–<50%, 3: ≥50%–<75%, 4: ≥75%). The final immunoscore was determined as the product of intensity and proportion scores, resulting in a range from 1 to 16. Cases were then classified as having low P4HB expression (score 1–8) or high P4HB expression (score 9–16) [14,15].

Statistical analysis

Data entry and analysis were performed using SPSS software version 23. Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized as mean, standard deviation, and range. When appropriate, comparisons between

categorical data were made using the Chi-square test or Fisher's exact test. A p-value of ≤ 0.05 was considered statistically significant.

RESULTS

Table 1 shows multivariate analysis, after adjusting for other variables, only concomitant CIS (OR: 47.9, 95% CI: 6.8 – 338.6, p<0.001) and high P4HB expression (OR: 112.9, 95% CI: 10.7 – 119.5, p<0.001) remained statistically significant independent predictors of high-grade urothelial carcinoma. Tumor size and muscle invasion were no longer significant in the multivariate model (p=0.63 and p=0.87, respectively). Age and sex were not included in the multivariate model as they were insignificant in the univariate analysis. These results suggest that concomitant CIS and high P4HB expression are strong independent predictors of high-grade urothelial carcinoma in this cohort.

Table 2 shows that most urothelial carcinoma patients were male (90.4%) and over 60 years of age (72.3%), with non-statistically significant differences between low-grade and high-grade tumors, as regards age (p=0.216) or sex distribution (p=0.513).

Table 3 demonstrates that high-grade urothelial carcinoma was significantly correlated with larger tumor size (>3 cm; p<0.001), higher frequency of muscle invasion (p<0.001), and greater prevalence of concomitant carcinoma in situ (CIS; p<0.001). Specifically, 67.6% of tumors >3 cm, 77.4% of muscle-invasive tumors, and 91.7% of cases with concomitant CIS were high-grade. In contrast, the distribution of histopathological type (papillary vs. non-papillary) did not differ significantly between grades (p=0.239).

Table 4 indicates that neither age nor sex was significantly correlated with P4HB expression levels among urothelial carcinoma patients. The proportion of high P4HB expression was similar across age groups (p=0.504) and between sexes (p=0.628), suggesting no demographic differences between low and high P4HB expression groups in this cohort.

Table 5 shows that high P4HB expression was significantly associated with larger tumor size (p<0.001), high tumor grade (p<0.001), and presence of muscle invasion (p<0.001). Specifically, 60.3% of tumors >3 cm, 79.2% of high-grade tumors, and 77.4% of muscle-invasive tumors exhibited high P4HB expression. In contrast, histopathological type (p=0.474) and concomitant CIS (p=0.235) were not significantly correlated with P4HB expression.

Figure (1) showing: Low-grade papillary non-muscle invasive urothelial carcinoma. A: Papillary fronds lined by malignant urothelial cells with minimal atypia(H&E, x400). B: Low P4HB expression(IHC, x 400). High-grade urothelial carcinoma. C: Cell nests of the malignant cells infiltrating the lamina propria. The malignant cells showed marked pleomorphism and hyperchromatism, (H&E, x400) D: high expression of P4HB(IHC, x400)

Figure 2 showing: Muscle invasive urothelial carcinoma (clear cell differentiation) A: malignant urothelial cells with clear cytoplasm (clear cell differentiation) infiltrating the muscle layer(H&E, x100) B: high expression of P4HB(IHC, x100) C: malignant cells with peripheral nucleus and abundant, clear cytoplasm with marked atypia and infiltrating muscularis propria(H&E, x400) D: high expression of P4HB(IHC, x100).

Table 1: Logistic regression analysis for prediction of high grade urothelial carcinoma

Variable	High grade urothelial carcinoma			
	Univariate		Multivariate	
	p-value	OR (95% CI)	p-value	OR (95% CI)
Age				
≤ 60 years		**Ref.**		
> 60 years	**0.219**	**1.78 (0.71 – 4.41)**	**-**	**-**
Sex				
Male		**Ref.**		
Female	**0.52**	**1.62 (0.38 – 6.9)**	**-**	**-**
Size				
≤ 3 cm		**Ref.**		**Ref.**
> 3cm	**<0.001**	**5.68 (2.08 – 15.5)**	**0.63**	**1.52 (0.28 – 8.45)**
Muscle invasion				
Absent		**Ref.**		**Ref.**
Present	**<0.001**	**8.26 (3.26 – 20.9)**	**0.87**	**0.84 (0.1 – 6.74)**
Concomitant CIS				
Absent		**Ref.**		**Ref.**
Present	**<0.001**	**13.84 (3 – 63.4)**	**<0.001**	**47.9 (6.8 – 338.6)**
P4HB expression				
Low expression		**Ref.**		**Ref.**
High expression	**<0.001**	**48.4 (12.5 – 186.5)**	**<0.001**	**112.9 (10.7 – 119.5)**

Table 2: Comparison between urothelial carcinoma grades as regards demographic data (n=94)

	Total (n=94)	Low grade (n=41)	High grade (n=53)	P-value
Age				
≤ 60 years	26 (27.7%)	14 (53.8%)	12 (46.2%)	
> 60 years	68 (72.3%)	27 (39.7%)	41 (60.3%)	0.216
Sex				
Male	85 (90.4%)	38 (44.7%)	47 (55.3%)	
Female	9 (9.6%)	3 (33.3%)	6 (66.7%)	0.513

Correlations done using Chi Square test

Table 3: Comparison between urothelial carcinoma grades as regards clinic-pathological data (n=94)

	Total (n=94)	Low grade (n=41)	High grade (n=53)	P-value
Size				
≤ 3 cm	26 (27.7%)	19 (73.1%)	7 (26.9%)	
> 3cm	68 (72.3%)	22 (32.4%)	46 (67.6%)	**<0.001**
Histopathological type				
Papillary carcinoma	70 (75.5%)	33 (47.1%)	37 (52.9%)	
Non-Papillary	24 (25.5%)	8 (33.3%)	16 (66.7%)	0.239
Muscle invasion				
Present	53 (56.4%)	12 (22.6%)	41 (77.4%)	
Absent	41 (43.6%)	29 (70.7%)	12 (29.3%)	**<0.001**
Concomitant CIS				
Present	24 (25.5%)	2 (8.3%)	22 (91.7%)	
Absent	70 (74.5%)	39 (55.7%)	31 (44.3%)	**<0.001**

CIS: Carcinoma in situ, Correlations done using Chi Square test

Table 4: Comparison between P4HB expressions as regards demographic data (n=94)

	Total (n=94)	Low expression (n=49)	High expression (n=45)	P-value
Age ***≤ 60 years*** ***> 60 years***	 26 (27.7%) 68 (72.3%)	 15 (57.7%) 34 (50%)	 11 (42.3%) 34 (50%)	 0.504
Sex ***Male*** ***Female***	 85 (90.4%) 9 (9.6%)	 45 (52.9%) 4 (44.4%)	 40 (47.1%) 5 (56%)	 0.628

Correlations done using Chi Square test

Table 5: Comparison between P4HB expressions as regards clinic-pathological data (n=94)

	Total (n=94)	Low expression (n=49)	High expression (n=45)	P-value
Size ≤ 3 cm > 3cm	 26 (27.7%) 68 (72.3%)	 22 (84.6%) 27 (39.7%)	 4 (15%) 41 (60.3%)	 **<0.001**
Histopathological type Papillary carcinoma Non-Papillary	 70 (75.5%) 24 (25.5%)	 38 (54.3%) 11 (45.8%)	 32 (45.7%) 13 (54.2%)	 0.474
Grade Low grade High grade	 41 (43.6%) 53 (56.4%)	 38 (92.7%) 11 (20.8%)	 3 (7.3%) 42 (79.2%)	 **<0.001**
Muscle invasion Present Absent	 53 (56.4%) 41 (43.6%)	 12 (22.6%) 37 (90.2%)	 41 (77.4%) 4 (9.8%)	 **<0.001**
Concomitant CIS Present Absent	 24 (25.5%) 70 (74.5%)	 10 (41.7%) 39 (55.7%)	 14 (58.3%) 31 (44.3%)	 0.235

CIS: Carcinoma in situ, Correlations done using Chi Square test

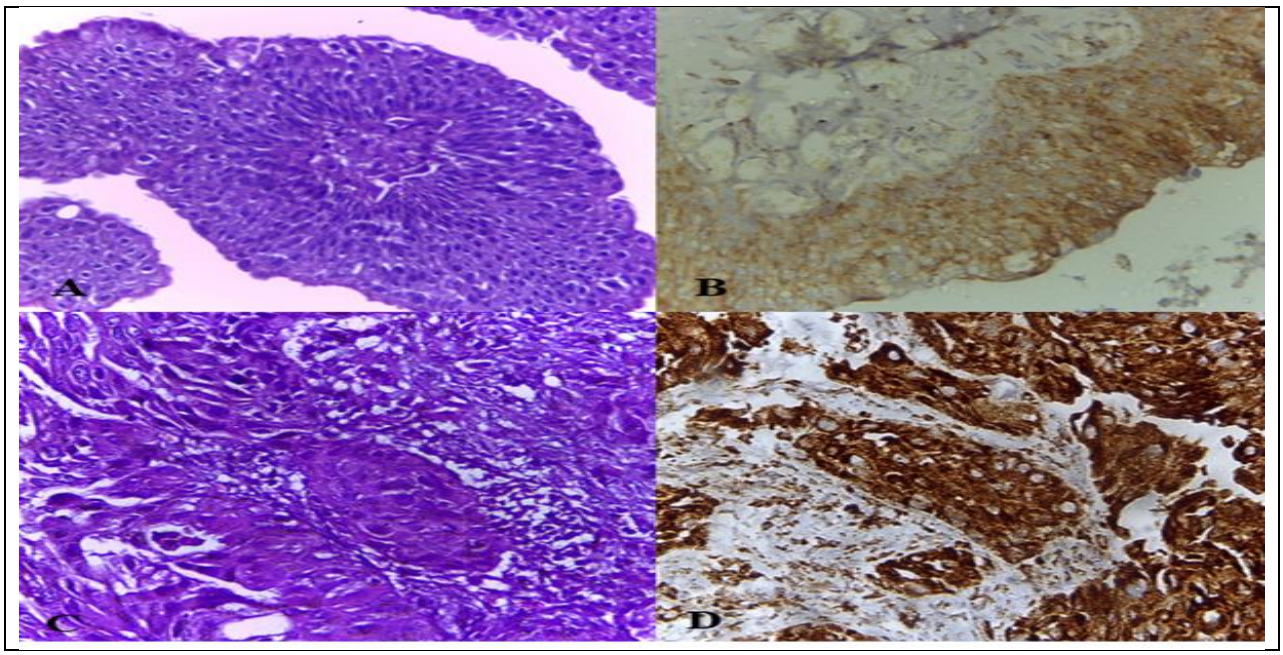

Figure (1): Low grade papillary non muscle invasive urothelial carcinoma A: Papillary fronds lined by malignant urothelial cells with minimal atypia(H&E, x400) B: low P4HB expression(IHC, x 400), High grade urothelial carcinoma C: cell nests of the malignant cells infiltrating lamina propria . The malignant cells showed marked pleomorphism and hyperchromatism, (H&E, x400) D: high expression of P4HB(IHC, x400)

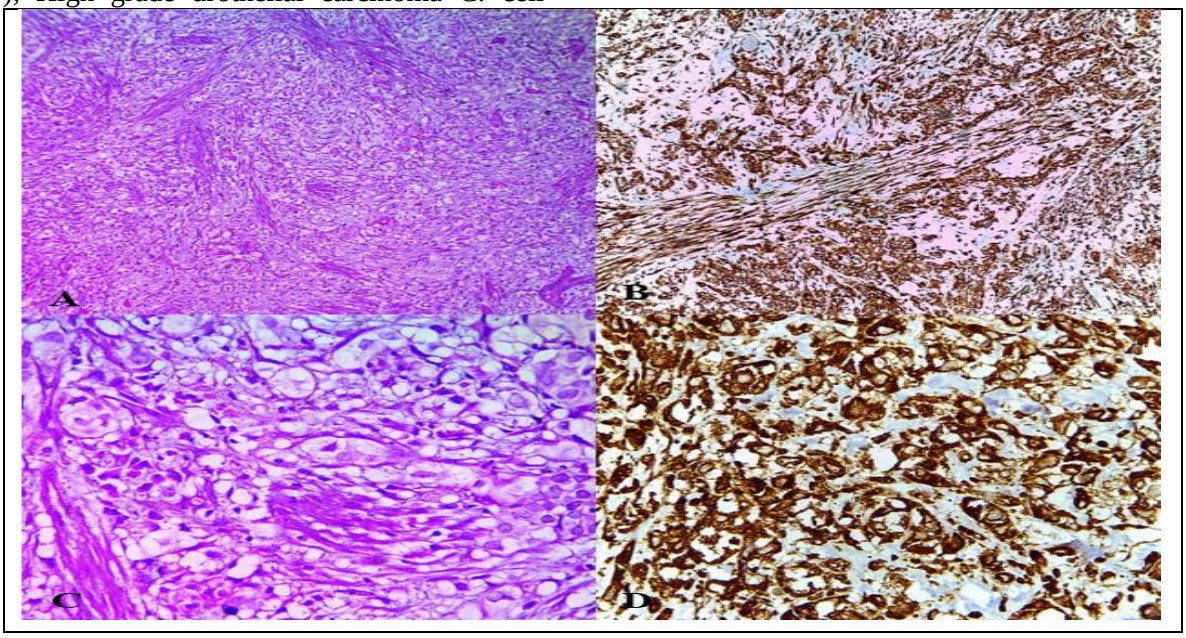

Figure 2: Muscle invasive urothelial carcinoma (clear cell differentiation) A: malignant urothelial cells with clear cytoplasm (clear cell differentiation) infiltrating the muscle layer(H&E , x100) B: high expression of P4HB(IHC, x100) C: malignant cells with peripheral nucleus and abundant, clear cytoplasm with marked atypia and infiltrating muscularis propria(H&E, x400) D: high expression of P4HB(IHC, x100).

DISCUSSION

Prolyl-4-hydroxylase subunit beta is a multifunctional enzyme belonging to the PDI family. This protein f=unctions as a molecular chaperone within the endoplasmic reticulum (ER), where it maintains ER homeostasis by facilitating the proper folding of proteins, preventing misfolded protein aggregation, and mediating the oxidation, reduction, and rearrangement of disulfide bonds. [9]. Previous research has demonstrated that silencing P4HB expression markedly reduces cell invasion and proliferation in bladder cancer. Additionally, P4HB has been associated with enhanced tumor cell proliferation, invasiveness, and metastatic capacity through its regulatory effects on hypoxia-inducible factor-1α (HIF-1α) and activation of the MAPK signaling cascade [16].

P4HB is believed to contribute significantly to the pathogenesis of urothelial carcinoma by facilitating tumor progression, enhancing resistance to stress-related apoptosis, and inhibiting apoptotic mechanisms. These mechanisms support tumor cell survival, proliferation, and metastasis [14]. However, there is limited information regarding the association between P4HB expression and clinicopathological characteristics in urothelial carcinoma. The current research aimed to analyze the prognostic value of P4HB and its correlation with clinicopathological features in urothelial carcinoma cases.

We compared demographic and clinicopathological data between low-grade and high-grade tumors. Tumor size statistically correlated with grade ($p<0.001$). Of tumors ≤3 Cm, 73.1% were low-grade and 26.9% were high-grade. Conversely, among tumors >3 cm, 32.4% were low-grade and 67.6% were high-grade. This agreed with Eroglu et al. [17], who observed that tumor measurements on CT urography are sensitive and specific in predicting high-grade tumors, and with Rahman et al. [18], who also reported a higher incidence of high-grade tumors among patients with tumor size >3.5 cm. In contrast, Lee et al. [19] found non-significant correlations between tumor size and time to overall progression ($p=0.108$), and Rosenkrantz et al. [20] reported no significant difference in tumor diameter between high and low grades. These variations across studies may result from differences in sample characteristics, tumor size thresholds, or imaging techniques.

Our findings demonstrated that papillary carcinoma was the most common histopathological type, comprising 47.1% low-grade and 52.9% high-grade cases. However, the distribution of histopathological types across grades was not statistically significant ($p=0.239$). This contrasts with the results of Xu et al. [21], who reported a significantly higher frequency of high-grade tumors in certain histological variants—such as squamous differentiation, glandular differentiation, nested, and micropapillary types—with a notable statistical correlation ($p=0.001$). Differences in histological subtypes, sample size, or population genetics might account for this discrepancy.

Muscle invasion was significantly more prevalent in high-grade tumors in our series ($p<0.001$): 22.6% of muscle-invasive tumors were low-grade, while 77.4% were high-grade. Among non-muscle-invasive tumors, 70.7% were low-grade and 29.3% were high-grade. This finding is consistent with the study by Hashmi et al. [22], who also established a significant correlation between muscle invasion and tumor grade ($p=0.006$).

We also observed that concomitant carcinoma in situ (CIS) was significantly more frequent in high-grade tumors ($p<0.001$): 91.7% of cases with concomitant CIS were high-grade compared to only 8.3% low-grade. This aligns with Shariat et al. [23], who reported that concomitant CIS is more common among high-grade tumors. However, Moschini et al. [24] did not obtain a significant relation between CIS and tumor grade ($p=0.119$), which could be explained by differences in patient selection, diagnostic criteria, or study design.

This study further evaluated P4HB immunohistochemical expression in non-muscle-invasive and muscle-invasive urothelial

carcinoma to explore its role in early detection and prognosis.

Tumor size was significantly related to P4HB expression ($p<0.001$). Among tumors ≤3 Cm, 84.6% had low P4HB expression, while only 15% had high expression. Conversely, tumors >3 cm showed low P4HB expression in 39.7% and high in 60.3% of cases. This result agrees with Soliman et al. [25], who found a significant correlation between tumor size and P4HB expression ($p=0.002$).

For tumor grade, a significant difference in P4HB expression was observed ($p<0.001$): 77.6% of low P4HB-expressing tumors were low-grade, while 93.3% of high P4HB-expressing tumors were high-grade. This finding is supported by Soliman et al. [25] ($p=0.00$) and Wang et al. [14] ($p=0.003$), both of whom demonstrated significant correlations between P4HB expression and tumor grade.

Muscle invasion was also strongly correlated with P4HB expression ($p<0.001$): 77.4% of muscle-invasive tumors had high P4HB expression, while only 22.6% had low expression. Among non-muscle-invasive tumors, high P4HB expression was present in only 9.8% of cases. Similar results were reported by Soliman et al. [25], who found high P4HB expression in 91.7% of muscle-invasive cases ($p=0.00$), and by Zou et al. [26], who also observed a significant association between high P4HB expression, muscle invasion, and advanced tumor stage ($p<0.001$).

We found no significant relationship between concomitant CIS and P4HB expression ($p=0.235$); among CIS cases, 41.7% showed low expression and 58.3% high expression. In line with our findings, Soliman et al. [25] observed that 33.3% of cases with concomitant CIS had high P4HB expression.

In our multivariate analysis, concomitant CIS and high P4HB expression emerged as strong independent predictors of high-grade urothelial carcinoma. This is supported by Wu et al. [27] and Zou et al. [26], who also identified high P4HB expression as a marker of poor prognosis and an independent predictor in urothelial carcinoma.

Furthermore, studies in other tumor types have reinforced the oncogenic and prognostic roles of P4HB. Zou et al. [28] found significantly higher P4HB expression in high-grade astrocytomas with poor prognosis. Xia et al. [29] observed that P4HB levels were significantly correlated with tumor grade, stage, number, and vascular invasion in hepatocellular carcinoma. Zhu et al. [30] associated P4HB expression with TNM staging and poor survival in clear-cell renal-cell carcinoma, and Zhang et al. [31] linked P4HB expression to age, depth of invasion, nodal metastasis, and chemotherapy response in gastric carcinoma.

The precise oncogenic mechanisms of P4HB remain to be fully elucidated. Xia et al. [29] suggested that P4HB may promote epithelial-to-mesenchymal transition (EMT) in hepatocellular carcinoma by downregulating glucose-regulatory protein 78. Zhou et al. [32] reported that P4HB knockdown induces apoptosis in colon-cancer cells by increasing reactive oxygen species and inhibiting STAT3 signaling. Ma et al. [33] showed that P4HB knockdown reduces β-catenin and Snail expression, implicating the β-catenin/Snail pathway in EMT modulation. These findings highlight the need for further studies to clarify the oncogenic role of P4HB in cancer development and progression.

A key strength of this study is the relatively large sample size, including 94 well-characterized cases of urothelial carcinoma, all with detailed clinicopathological data and immunohistochemical analysis of P4HB expression. Standardized pathological evaluation and robust statistical analysis enhance the reliability of the results. The study comprehensively correlates P4HB expression with multiple clinically relevant parameters, providing novel insights into its prognostic significance in bladder cancer within the Egyptian population.

The main limitations of this study are its retrospective single-center design and the lack of long-term clinical follow-up data, which may limit the ability to assess survival outcomes. Potential selection bias may also exist due to

excluding cases with insufficient tissue or missing data. Furthermore, the study did not include molecular analyses to confirm mechanistic pathways, and the findings may not be fully generalizable beyond the studied population.

CONCLUSION

This study demonstrates that elevated P4HB expression correlates with increased tumor size, higher grade, and advanced stage in urothelial carcinoma. Detection of P4HB in low-grade and early-stage tumors highlights its value for early diagnosis, while its strong association with aggressive features supports its use as a prognostic biomarker. Overall, assessing P4HB expression may aid in predicting tumor progression and could inform future therapeutic strategies in bladder cancer management.

Conflict of Interest or financial disclosure: No potential conflict of interest or financial funding to be reported by the authors.

Availability of Data: Data supporting the findings of this study are accessible from the corresponding author upon reasonable request.

Author Contribution: T.A.A. led the conceptualization of the research, conducted the primary pathological investigations, and was responsible for manuscript preparation and correspondence. M.I.A. contributed to the study design and provided critical supervision throughout all research stages. M.M.A. assisted with pathological data analysis and interpretation, while A.A.M. offered valuable insights during the drafting and revision of the manuscript. All authors reviewed and approved the final version.

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