THE ROLE OF PROSTEIN (P501S) AND GATA3 IMMUNOSTAINING IN DIFFERENTIATING POORLY DIFFERENTIATED PROSTATIC CARCINOMA FROM HIGH GRADE UROTHELIAL CARCINOMA

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ABSTRACT

Background: Prostatic cancer (PC) is now recognized as one of the common medical problems facing the male population accounting for 33% of all malignant tumors**.** In Egypt, cancer bladder constitutes 30% of all cases attended at the National Cancer Institute with an overall incidence rate of 13.5/100,000 individual. The histological distinction between poorly differentiated prostatic adenocarcinoma and high grade urothelial carcinoma (UC) can be difficult. This distinction is important due to prognostic and therapeutic consideration.

Aim of work: To differentiate between poorly differentiated prostatic adenocarcinoma and high grade UC immunohistochemically, using prostein (P501s) and GATA3.

Methods: Prostein (P501s) and GATA3 expressions were retrospectively analysed by immunohistochemistry in two groups. The first group consisted of 42 paraffin-embedded specimens of poorly differentiated prostatic adenocarcinoma and 45 paraffin-embedded specimens of high grade urothelial carcinoma (documented group)**.** The second group consisted of 8 cases of prostatic biopsies and 5 cases of bladder biopsies in which the origin of the carcinoma couldn't be assessed (problematic group). The expressions were correlated with clinicopathological variables.

Results: The immunoexpression of prostein was positive in 40/ 42 (95.3%) of poorly differentiated prostatic adenocarcinoma while showed negative staining in 100% of cases of high grade urothelial carcinoma (documented group) with a statistically highly significant correlation ($p<0.0001$). There is statistically significant an inverse correlation between prostein immunoexpression and the Gleason score for poorly differentiated prostatic adenocarcinoma (documented group) (p value = 0.02). GATA3 was positive in 44/45 (97.8%) of high grade urothelial carcinoma while negative in 100% of poorly differentiated prostatic adenocarcinoma (documented group) with a statistically highly significant correlation ($p <0.001$). There is an inverse correlation between the depth of invasion of high grade urothelial carcinoma and GATA3 immunoexpression and it was statistically significant ($p =0.01$). Prostein sensitivity and specificity for poorly differentiated prostatic adenocarcinoma (documented group) was 94.5% and 100% respectively, while the sensitivity of GATA3 for high grade urothelial carcinoma (documented group) was 88.9% and specificity was 100%.

Conclusions: Prostein (P501s) and GATA3 are useful markers in differential diagnosis between poorly differentiated prostatic adenocarcinoma and high grade urothelial carcinoma.

Keywords: Prostein(P501s) – GATA3 – urothelial carcinoma of urinary bladder – prostatic carcinoma Immunohistochemistry (IHC)

INTRODUCTION

Prostatic cancer (PC) is the most common malignancy in men, and the second leading cause of deaths in USA among men [1]. In Egypt, PC incidence according to the National Population-Based Registry Program in the period from 2008– 2011 was 4.2% among male cancers [2].

It is so difficult to determine the origin of the carcinoma in the cases of poorly differentiate prostatic adenocarcinoma and high grade urothelial carcinoma. The distinction between them is important due to prognostic and therapeutic consideration

as hormone therapy may be used in the treatment of prostatic adenocarcinoma but chemotherapy is used in the treatment of urothelial carcinoma [3].

The pathologic distinction between poorly differentiate prostatic adenocarcinoma involving the urinary bladder and high-grade urothelial carcinoma (UC) infiltrating the prostate can be difficult. The prostatic gland can be invaded by UC by direct invasion into the prostatic stroma (stageT4) or by the extent of the tumor through the prostatic ducts [4].The involvement of the urinary bladder by prostate adenocarcinoma may occur as a metastasis or by direct invasion and it represents 12% of all secondary bladder tumors and it is the second most common origin of bladder tumor [5].

It is very useful to use immunophenotyping in the diagnosis of the primary site of the tumor especially in poorly differentiated form [6]. Among the markers used to differentiate between urothelial and prostate cancers, prostate-specific antigen (PSA) and prostate-specific acid phosphatase (PSAP). These markers are the most commonly used to find the prostatic origin of tumors; however, their expression is significantly decreased in poorly differentiated prostatic cancers. Alpha-methylacyl-CoA-racemase (AMACR) is considered a beneficial marker for the diagnosis of prostatic carcinoma but it is also expressed in urothelial cancers. So, it is not useful in distinguish between them [6].

Among the newer markers, prostein (P501s) is a prostate specific 553 amino acid protein. It is confined to the Golgi apparatus which is expressed in normal prostate, benign prostatic hyperplasia, primary prostatic tumors and metastatic prostate carcinoma. Owing to its specificity for the prostatic tissue, prostein has an important role in the diagnosis of the advanced prostatic carcinoma with distant metastases [7].

Prostein (P501s) expression is not noticed in the transitional epithelium or any other tissues. Therefore it has been shown to have excellent specificity in the differentiation between poorly differentiated prostatic adenocarcinoma and high grade urothelial carcinoma [8]

To establish urothelial differentiation, high molecular weight cytokeratin (HMWCK, clone 34βE12), thrombomodulin, CK 7 and CK 20 are commonly used in clinical practice. But, they are not specific for UC. Among the markers of urothelial origin, uroplakin III is a highly specific for UC but with moderate sensitivity for UC [6].

Among the newer marker, GATA 3 is a multifunctional transcription factor. It plays an important role in promoting, directing the cell proliferation, differentiation and development. GATA3 has been used for detection of tumors with urothelial origin. Since then it has been useful in the distinction between urothelial carcinoma versus prostatic adenocarcinoma. [9]

The appearance of GATA3 is down-regulated in transitional neoplasms, more than non-neoplastic urothelial tissues, and in high grade urothelial carcinoma with or without muscle invasion more than low grade urothelial carcinoma also with superficial carcinoma So, loss of GATA3 is correlated with increased of cell invasion and migration [10]

MATERIALS AND METHODS

Materials

The type of this study was comparative retrospective cross sectional study and the sampling method was random non-probability sample. The materials of this study were divided in two groups. The first group consisted of 42 paraffin-embedded specimens of poorly differentiated prostatic adenocarcinoma and 45 paraffin-embedded specimens of high grade urothelial carcinoma (documented group). The second group consisted of 8 cases of prostatic biopsies and 5 cases of bladder biopsies in which the origin of the carcinoma couldn't be assessed (problematic group) selected from the archive of Pathology Department, Faculty of Medicine, Zagazig University (30 cases) and some private laboratories (70

cases) in the period from 2014 to 2016. The clinical data concerning age was obtained from archive files of the corresponding departments. The cases of poorly differentiated prostatic adenocarcinoma and high grade urothelial carcinoma were included in this study. The criteria for the selection of high grade urothelial carcinoma were increase of the nuclear size, nuclear pleomorphism and frequent mitosis according to WHO classification 2016. The presence of carcinoma in situ in the surface of the bladder biopsies was confirming our documented group of high grade urothelial carcinoma The cases of prostatic carcinomas and high grade urothelial carcinoma who received neu-adjavant chemotherapy, radiotherapy, or hormonal therapy were excluded. For prostatic biopsies: 33 cases were obtained by transurethral resection specimens (TURS), (11) cases by ultrasound-guided transrectal biopsies (TRUS), (6) cases by prostatectomy. For bladder biopsies: (26) cases were obtained by cystoscopy, (5) cases by cystectomy, (8) cases by radical cystoprotatectomy and (11) cases by TURS. Hematoxylin and eosin (H&E) stained sections were examined by binuclear light microscope; grading of poorly differentiated prostatic adenocarcinoma was based on Gleason score [11]. The cases of poorly differentiated prostatic adenocarcinoma were divided into Gleason score 8, 9 and 10. The pathologic depth of invasion of high grade UC was defined according to the TNM staging system tumors [12].

Methods

Immunohistochemistry

Immunohistochemical staining was carried out using indirect streptoavidin-biotin immunoperoxidase technique. Tissue sections (3–5 µm) were deparaffinized in xylene and rehydrated in graded alcohol. Slides were incubated for 10 minutes in o.3 % hydrogen peroxide in absolute methanol to block endogenous peroxidase activity. Antigen retrieval was performed using Dako target retrieval solution(pH 6.0) (Dako, CA, USA).The slides were then incubated for 60 minutes at room temperature using 2-3 drops of primary antibody prostein (P501) was placed on each slide (rabbit monoclonal antibody Synthetic peptide corresponding to human SLC45A3 at N- terminal (KLH). (Dilution 1:500, Clone 10E3, Code M3615, Biocare Medical, USA) and GATA3: A mouse monoclonal antibody (Dilution 1:100, clone L50-823, Biocare Medical, USA) then washed with two changes of PBS, stained again with secondary antibody for 15 minutes at room temperature. The slides were incubated in humidity chamber overnight at 2-8 C. Diamminobenzidin (DAB) substrate was added to tissue sections; incubated for 5-10 minutes then slides were immersed in a bath of Mayer's hematoxylin (M.H); incubated for (2-5) minutes.

Positive control for prostein (P501): Normal prostatic glands used as internal control. Positive control for GATA3: Normal urothelium or the basal cells of benign prostatic glands. Negative control is achieved by omitting the step of primary antibody.

Immunohistochemical evaluation

Interpretation of immunohistochemical staining of prostein:

Positive reaction for prostein was seen as granular cytoplasmic staining. The extent for prostein expression divided into 4-point scoring system according to the percentages of stained malignant cells: Score 0 (none %), score 1 (< 5 %), score 2 (5-25 %%) and score 3 (> 25%). Intensity of prostein stained malignant cells was scored in to negative (0), weak (1+), moderate (2+) and strongly (3+) [13].

Interpretation of immunohistochemical staining of GATA3:

Positive reaction for GATA3 was seen as nuclear staining. The extent of GATA3 expression divided into 5-point scoring system according to the percentages of stained malignant cells: Score 0 (< 5 %), score 1 (5-25%) and score 2 (26-50 %) while score 3 (51-75%) and score 4 (76-100%) [14]. The GATA3 intensity stained malignant cells was scored in to negative , weak positive (1+), moderate positive (2+) or strong positive staining (3+) [15].

Statistical analysis:

All data were collected, tabulated and statistically analyzed using SPSS 20 for windows (IBM Corp .Armonk. 2011). Categorized data were compared using the Chi-square ($\chi 2$) test while Fisher's exact test was used if expected count < 5. All tests were two sided. P value < 0.05 was considered statistically significant (S) and p value >0.05 was considered none statistically significant (NS).

RESULTS

The cases of this study were divided into two groups. The first group consisted of 42 paraffin-embedded specimens of poorly differentiated prostatic adenocarcinoma and 45 paraffin-embedded specimens of high grade urothelial carcinoma (documented group). The second group consisted of 8 cases of prostatic biopsies and 5 cases of bladder biopsies that were difficult in their diagnosis in which the origin of the carcinoma couldn't be assessed (problematic group).

Regarding to age distribution among the studied cases, 56% of cases of the poorly differentiated prostatic adenocarcinoma were in 6^{th} decade, while 40% of cases of high grade UC were in 7^{th} decade. The studied cases of poorly differentiated prostatic adenocarcinoma associated with mean age (66.5) while urothelial carcinoma associated with mean age (68.9).

The histological classification of the cases from prostatic biopsies was diagnosed as 42 cases (84%) were diagnosed as poorly differentiated prostatic adenocarcinoma (documented group) and 8 cases (16%) (Problematic group) which were difficult in their diagnosis. According to Gleason score for documented group 26.2 %, 23.8 % and 50 % of the cases were graded as 8, 9 and 10, respectively. The histologic classification of the cases from bladder biopsies 45 cases (90%) were diagnosed as high grade urothelial carcinoma (documented group) and 5 cases (10%) of cases (problematic group). The pathologic depth of invasion of high grade urothelial carcinoma (documented group) was divided in to 2 cases (4.4%) were pT1, 28 cases (62.2%) were pT2, 12 cases (26.7%) were pT3 and 3 cases (6.7%) were pT4.

A- Immunohistochemical results of prostein staining: 95.3% of studied cases of poorly differentiated prostatic adenocarcinoma showed positive prostein immunoexpression (52.4% of cases were score 3) while all cases of high grade UC (documented group) showed negative prostein immunoexpression. There is an inversely statistically significant correlation between prostein expression of poorly differentiated prostatic adenocarcinoma and histological Gleason grade (p=0.02) where prostein expression decreased with increasing the Gleason grade and score. The correlation between prostein expression in poorly differentiated PC and high grade UC showed statistically highly significant correlation (p. value < 0.0001).

B- Immunohistochemical results of GATA3 staining: GATA3 immunoexpression showed positive staining in 97.8 % of high grade UC while all cases of poorly differentiated prostatic adenocarcinoma (documented group) showed negative GATA3 staining. The correlation between GATA3 immunoexpression in poorly differentiated PC and high grade UC showed a statistically highly significant difference (p. value <0.001). The correlation between GATA3 immunoexpression and pathologic depth of invasion of high grade urothelial carcinoma were statistically highly significant (p. value <0.001).

In this study prostein has a sensitivity of 94.5% and a specificity of 100% for poorly differentiated prostatic adenocarcinoma while the sensitivity of GATA3 was 88.9 %, and specificity was 100% for high grade urothelial carcinoma (documented group).

Table 1: Classification of the studied cases

1st group	**Documented group**	Poorly differentiated prostatic adenocarcinoma (42 cases)
		High grade Urothelial carcinoma (45 cases)
2nd group	**Problematic group**	Problematic cases from prostatic biopsies (8 cases)
		Problematic cases from urinary bladder biopsies (5 cases)

Table 2: The clinical and pathological characteristics of studied prostatic cases

Variables	**Prostatic cases (50)**
Age	
Mean ± SD	66.5±8.1, range (40-80)
Histological type	
Poorly differentiated prostatic adenocarcinoma (documented group)	42 (84%)
Problematic group	8 (16%)
Grading according to Gleason score (documented group 42 cases)	
Gleason score 8	11 (26.2%)
Gleason score 9	10 (23.8%)
Gleason score 10	21 (50%)

Table 3: The clinical and pathological characteristics of studied urinary bladder cases

Variables	**Urinary bladder cases (n=50)**
Age	
Mean ± SD	68.9±9.2 , range (45-83)
Histological type	
High grade urothelial carcinoma (documented group)	45 (90%)
Problematic group	5 (10%)
Pathologic depth of invasion (pT) of high grade urothelial carcinoma (documented group 45 cases)	
pT1	2 (4.4%)
pT2	28 (62.2%)
pT3	12 (26.7%)
pT4	3 (6.7%)

Table 4: The correlation between histological grade and the intensity of prostein immunoexpression of the poorly differentiated prostatic adenocarcinoma (documented group)

Prostein immunoexpression	Gleason score of prostatic adenocarcinoma						X^2	P
	Gleason score 8		Gleason score 9		Gleason score 10			
	No	%	No	%	No	%		
Negative	0	0.0	0	0.0	2	9.5	0.7	0.02* (S)
Weak	1	9	2	20	4	19		
Moderate	3	27	2	20	9	23.8		
Strong	7	64	6	60	6	47.6		
Total	11	100	10	100	21	100		

χ^2: Chi-square test., (S) significant; p< 0.05 is significant.

Table 5: The immunoexpression of prostein in the documented group

Characteristics	N	Prostein expression		χ^2	p-value
		positive	negative		
Histological type					
orly differentiated prostatic adenocarcinoma	42	40 (95.3%)	2(4.6%)	76.053	0.0001 (HS)
igh grade urothelial carcinoma	45	0 (0%)	45 (100%)		

χ^2: Chi-square test.; HS: Highly significant p< 0.05 is significant

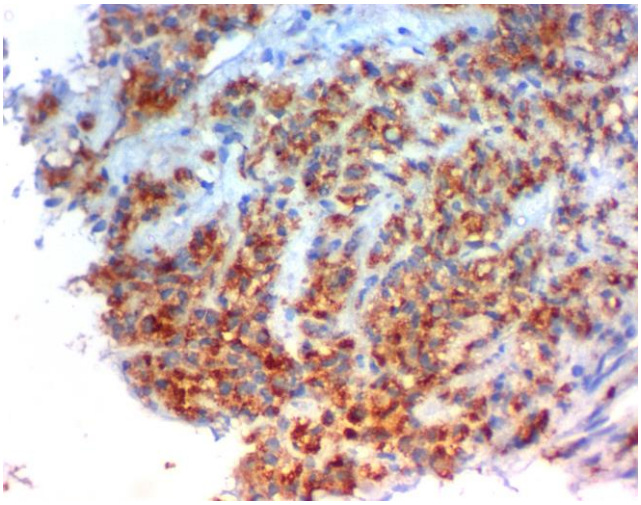


Figure 1: Poorly differentiated prostatic adenocarcinoma (documented case) showing strong positive granular cytoplasmic brown prostein stain score 3 (prostein immunoexpression and Mayer's hematoxylin x 400).

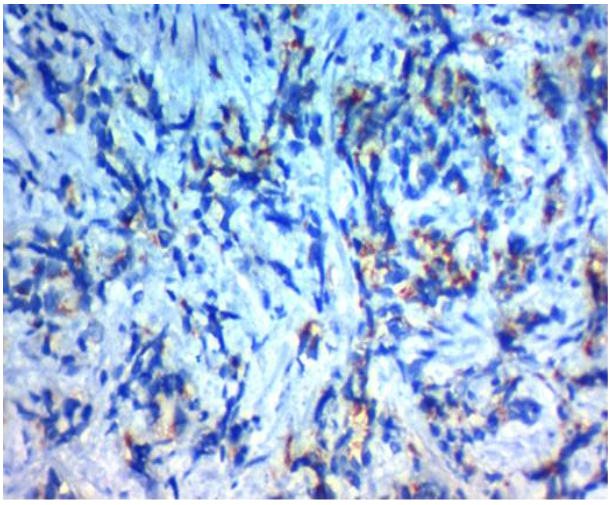


Figure 2: Poorly differentiated prostatic adenocarcinoma (documented case) showing weak positive granular cytoplasmic brown prostein stain score 2 (prostein immunoexpression, Mayer's hematoxylin x 400).

Table 6: The correlation between pathologic depth of invasion (pT) of high grade urothelial carcinoma (documented group) and the intensity of GATA3 immunoexpression

Pathologic depth of invasion (pT)	No	GATA3 immunoexpression								X^2	P
		Negative		Weak		Moderate		Strong			
		No	%	No	%	No	%	No	%		
pT1	2	0	0	0	0	0	0	2	100	61.1	<0.001 ** (HS)
pT2	28	0	0	0	0	10	35.7	18	64.2		
pT3	12	1	8.3	1	8.3	4	33.3	6	50		
pT4	3	0	0	2	66.6	1	33.3	0	0		
Total	45	1	2.2	3	6.6	15	33.3	26	57.7		

χ^2: Chi-square test.; HS: Highly significant p< 0.05 is significant

Table 7: The immunoexpression of GATA3 in the documented group

Characteristics	N	GATA3 expression		χ^2	p-value
		positive	negative		
Histological type					
Poorly differentiated prostatic adenocarcinoma	42	0 (0%)	42(100%)	53.5	0.001 (HS)
High grade urothelial carcinoma	45	44 (97. 8%)	1 (2.2 %)		

χ^2: Chi-square test.; HS: Highly significant p< 0.05 is significant

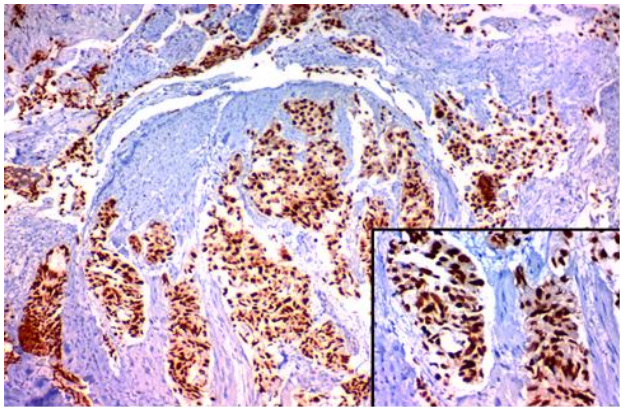


Figure 3: High grade urothelial carcinoma with muscle invasion (documented case) showing strong positive nuclear GATA3 staining score 4 (GATA3 immunoexpression, Mayer's hematoxylin x 100).

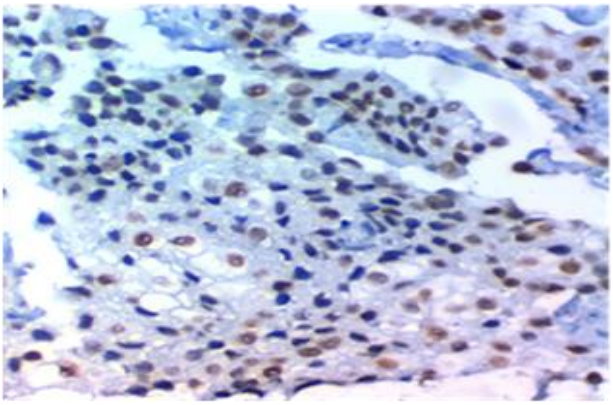


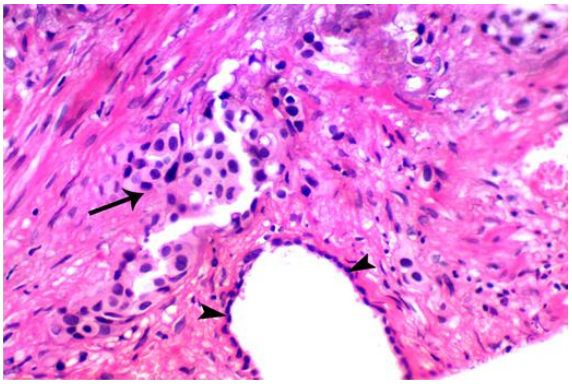
Figure 4: High grade urothelial carcinoma (documented case) showing moderate positive nuclear GATA3 staining score 3 (GATA3 immunoexpression, Mayer's hematoxylin x 400)

Table 8: Values of sensitivity and specificity of prostein in poorly differentiated prostatic adenocarcinoma and GATA3 in of high grade urothelial carcinoma (documented group)

IHC	Sensitivity	Specificity	PPV	NPV	Accuracy
Prostein	94.5%	100%	100%	95%	96%
GATA3	88.9%	100%	100%	90	90%

Table 9: The final diagnosis of the problematic group after the immunohistochemical results

Biopsy	Prostein immunoexpression				GATA3 immunoexpression				final diagnosis
	Positive		Negative		Positive		Negative		
	No	%	No	%	No	%	No	%	
Prostatic biopsies (8 cases)	5	62.5%	0	0	0	0	5	62.5%	**Poorly differentiated prostatic adenocarcinoma (5 cases)**
	0	0	2	25%	2	25%	0	0	**Metastatic high grade urothelial carcinoma (2 cases)**
	1	12.5%	0	0	1	12.5%	0	0	**Collision tumor (one case)**
Total	6	75%	2	25%	3	37.5%	5	62.5%	
urinary bladder biopsies (5 cases)	2	40%	0	0	0	0	2	40%	**Metastatic adenocarcinoma from prostatic (2 cases)**
	0	0	2	40%	2	40%	0	0	**High grade urothelial carcinoma (2 cases)**
	1	20%	0	0	1	20%	0	0	**Collision tumor (one case)**
Total	3	60%	2	40%	3	60%	2	40%	



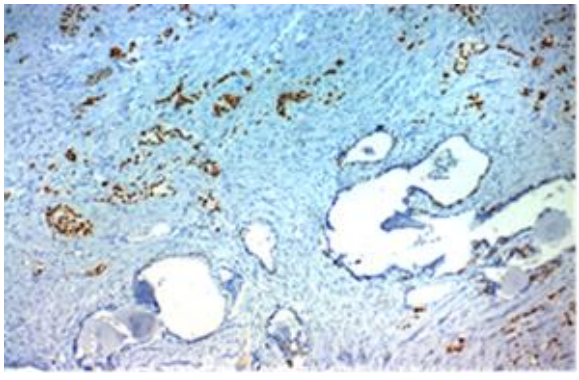


Figure 5: Prostatic biopsy (TURs) of a problematic case showing sheets of malignant epithelial cells with deeply stained hyperchromatic nuclei and eosinophilic cytoplasm (arrow) infiltrate prostatic tissue. An adjacent atrophic prostatic acini (arrow heads) (H & E x 400).

Figure 6: The previous problematic case showing positive nuclear brown GATA3 staining denoting presence of high grade urothelial carcinoma infiltrating the prostatic tissue (GATA3 immunoexpression, Mayer's hematoxylin x 100).

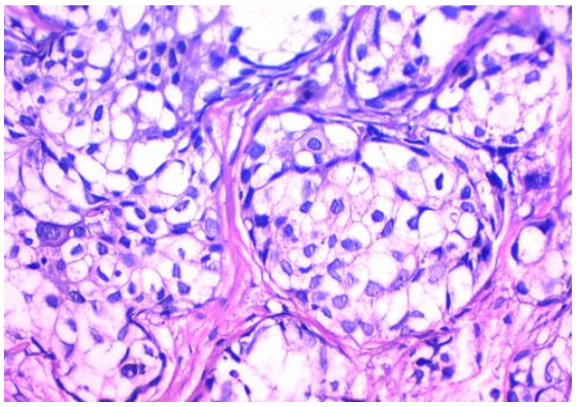


Figure 7: TURs of the problematic case showing undetermined carcinoma with sheets of malignant cells with clear cytoplasm (H&E x 400)

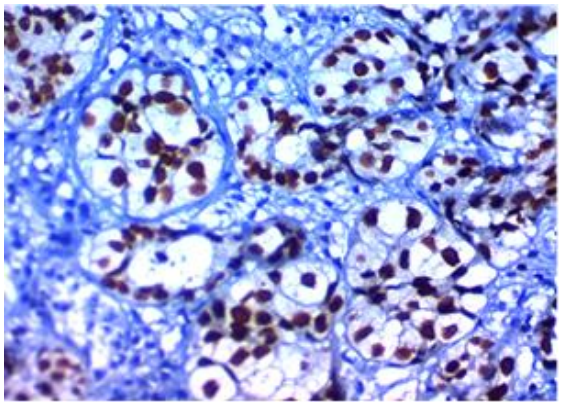


Figure 8: The previous problematic case showing positive nuclear GATA3 staining denoting presence of urothelial carcinoma (GATA3 immunoexpression, Mayer's hematoxylin X400)

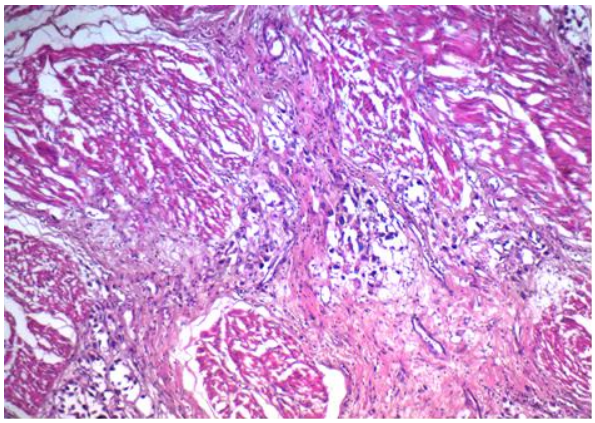


Figure 9: Cystoscopic biopsy of a problematic case showing sheets of malignant epithelial cells dissecting muscle fibers of the urinary bladder (H &E x 100)

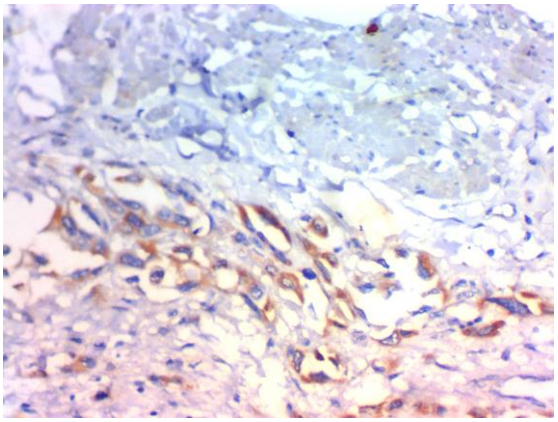


Figure 10: The previous case showing positive cytoplasmic brown prostein staining denoting presence of invasion by poorly differentiated prostatic adenocarcinoma to urinary bladder (prostein immunoexpression , Mayer's H x 400)

Table 10: Correlation between prostein and GATA3 immunoexpression between the documented group and problematic group

Diagnosis	prostein				GATA3				χ^2	p
	Negative		Positive		Negative		Positive			
	No.	%	No.	%	No.	%	No.	%		
Poorly differentiated prostatic adenocarcinoma (n=42)	**0**	**0.0**	**42**	**100.0**	**42**	**100.0**	**0**	**0.0**		
Problematic group (n=8)	**2**	**25%**	**6**	**75 %**	**5**	**62.5**	**3**	**37.5**	**87.3**	**<0.001** HS
Total (n=50)	**2**	**4%**	**48**	**96%**	**47**	**94%**	**3**	**6%**		
High grade urothelial carcinoma (n=45)	**45**	**100.0**	**0**	**0.0%**	**1**	**1 2.2**	**44**	**97.8%**		
Problematic group (n=5)	**3**	**60**	**2**	**40%**	**2**	**40**	**3**	**60**		
Total (n=50)	**48**	**96%**	**2**	**4%**	**3**	**6%**	**47**	**97%**	**97.3**	**<0.001** HS

: χ^2: Chi-square test.; HS: Highly significant
p< 0.05 is significant

DISCUSSION

Due to the anatomical proximity existing between the prostate and the urinary bladder, a possible invasion of the prostatic gland by urothelial carcinoma of the urinary bladder or infiltration of the urinary bladder by a prostatic carcinoma is commonly encountered. In the case of well-differentiated neoplasms, the histological assessment based on a routine hematoxylin and eosin staining is adequate for a right diagnosis. [16].

It is frequently difficult to know the histologic type of poorly differentiated tumor as either a prostatic or urothelial carcinoma or a collision tumor formed of urothelial carcinoma and prostatic adenocarcinoma. In addition, the difficult in accurate distinction is increased when there is only incomplete tissue available, such as in needle biopsies, cell blocks with only small foci of carcinoma [16].

It is important to differentiate between these tumors due to the differences in the management modalities and patient outcomes. [17].

Prostein (P501s is a 553-amino acid protein which contains 11 potential transmembrane spanning domains. The protein is localized to the Golgi apparatus of the cell; it is used as prostate specific marker [18].

In the current study, poorly differentiated prostatic adenocarcinoma (documented group) showed positive granular cytoplasmic prostein staining in 95.3% of cases and showed negative staining in 4.7% of cases. From this view, our results close to Kalos et al. [18], Yin et al. [13], Srinivasan and Parwani [6] and Queisser et al.[19], they found that prostein immunoexpression was positive in 94% , 94.1, 95.7% and 96% of cases of prostate adenocarcinoma respectively. In our results, prostein immunoexpression was negative in all cases of high grade urothelial carcinoma (documented group). This is in agreement with Mohanty et al. [20] but in contrast to

Chuang et al. [7] who found that 6% of high grade UC showed focal weak positivity for prostein. This difference is due to change in interpretation of the markers with us. In this study there is statistically highly significant difference (p. value <0.0001) between the expression of prostein in poorly differentiated prostatic adenocarcinoma and high grade urothelial carcinoma (documented group). Therefore, prostein is very helpful in differentiating between poorly differentiated PC from high grade UC (documented group). Our results agreed with Srinivasan and Parwani[6] , Mohanty et al. [20] and Oh et al. [4], they concluded that prostein was helpful in differentiation between poorly differentiated prostatic adenocarcinoma from high grade urothelial carcinoma. In our study , the correlation between histological grade according to Gleason score and the intensity of prostein immunoexpression for the cases of poorly differentiated prostatic adenocarcinoma was significant (p value = 0.02) in agreement with Paner et al.[21] who found that the expression of prostein correlated inversely with Gleason scores which high Gleason score showed low prostein expression (p < 0.001). In contrast with Yin et al. [13], Srinivasan and Parwani [6] and Oh et al. [4] they found that not significant correlation between the prostein intensity and the Gleason grade of the prostatic tumor. In our study, prostein has a sensitivity of 94.5% and a specificity of 100% in the poorly differentiated prostatic adenocarcinoma (documented group). This finding was in agreement with Srinivasan and Parwani [6], who proved that prostein sensitivity was 95.7% and 100% specificity. In addition, our present study is close to Oh et al. [4] who found that the prostein sensitivity was 93.7% and 100% specificity. This means that prostein had excellent specificity and sensitivity in differentiating between poorly differentiated prostatic adenocarcinoma and high grade urothelial carcinoma.

GATA3 is necessary for the growth and differentiation in many tissues as urothelial tissue and mammary glands of the breast. GATA3 is used to improve diagnostic accuracy and outcomes prediction in advanced UC [22]. In the current study, GATA3 immunoexpression showed positive nuclear reactivity in 97.8% of cases of high grade urothelial carcinoma (documented group) except only one case (2.2%) was negative for GATA3 immunoexpression which had squamous differentiation. This results were close to Ordo´n˜ez et al.[24] , Zheng and Blobel[23] and Mohanty et al. [20] they found that GATA 3 immunoexpression was also positive in 95% 99% and 100% of cases of urothelial carcinoma respectively. But our results were slightly different from Chang et al. [15] and Miettinen et al. [25] they found that most of urothelial carcinomas 89% and 90% showed positive for GATA3 immunoexpression respectively. In our study, we found that all cases (100%) of poorly differentiated prostatic adenocarcinoma (documented group) were negative for GATA3 immunoexpression in agreement with Higgins et al. [26] who proved that none of 257 cases of prostatic adenocarcinoma showed positive GATA3 immunoexpression but in contrast to Miettinen et al. [25] who founded that GATA 3 was focally positive in only 2.1% (2 of 95) of cases of prostatic adenocarcinoma. In the present study, there is highly significant difference (p. value <0.001) between poorly differentiated PC and high grade UC for GATA3 immunoexpression. This result was similar to Higgins et al. [26], Chang et al. [15] , Mohammed et al. [27] and Oh et al. [4]. Therefore,

GATA3 is very helpful marker in differentiation between high grade UC and poorly differentiated PC. In the present study, the specificity of GATA3 was 100% % in high grade urothelial carcinoma (documented group). These finding are close to Chang et al. [15] and Mohammed et al. [27]. In the present study, the sensitivity of GATA3 was 88.9% for urothelial carcinoma. This finding was close to but slightly higher than Higgins et al. [26] Chang et al.[15] , and Oh et al.[4] who stated that the sensitivity of GATA3 was 81.9%, 84.8% and 81.6% in UC respectively . They proved that GATA3 is the best in sensitivity for high grade UC. In our results GATA3 showed strong positive in 100% pT1 high grade urothelial carcinoma, 64% pT 2 high grade invasive urothelial carcinoma and weak expression in 66.6 in pT 4. This means decrease the expression of GATA 3 with increasing the pathologic depth of invasion.

In the present study, prostein and GATA3 can differentiate between the origins of the tumor of the problematic cases which were taken from prostatic biopsies (8 cases). Five cases from the eight problematic cases (62.5%) showed positive granular cytoplasmic prostein immunoexpression and negative for GATA3 immunoexpression, diagnosed as primary poorly differentiated prostatic adenocarcinoma. Two cases from the eight problematic cases (25%) of cases showed positive nuclear GATA3 staining and negative prostein staining were diagnosed invasive high grade urothelial carcinoma that metastasizes to the prostatic tissue. Only one problematic case (12.5%) was positive for both prostein and GATA3 diagnosed as collision tumor which contain both poorly differentiated PC and high grade UC which detected on a core needle biopsy from the prostatic gland.

In addition, the five problematic cases that were taken from bladder biopsies were diagnosed by using prostein and GATA3. Two cases from the five problematic cases (40%) were positive GATA3 and negative for prostein; diagnosed as primary high grade UC. The histopathology picture of the other two (2/5) problematic cases (40 %) showed penetration of malignant epithelial cells with variable areas some has major glandular shape and other areas of poorly differentiated cells supposed to be of prostatic origin which invade the bladder wall. The immunohistochemical analysis of these two cases showed positive prostein immunoexpression and negative nuclear GATA3 immunoexpression, diagnosed as metastatic poorly differentiated PC. Only one problematic case from the five problematic case (20%) taken by radical cystoprostatectomy for removal the carcinoma of urinary bladder but we found the presence of a second primary tumor in the prostate which present in its poorly differentiated picture. This problematic case showed a positivity for both prostein and GATA3 diagnosed (collision tumor) which formed of poorly differentiated PC and high grade UC.

In conclusions, our results revealed that prostein and GATA3 helpful in differentiation between high grade urothelial carcinoma and poorly differentiated prostatic adenocarcinoma. It is possible to find the collision tumor between UC and PC. So it must always be careful, mainly when the tumors presented in the poorly differentiated picture. It is better to do comprehensive research and include a greater number in another prospective study with follow up to detect other characters of prostein and GATA 3 expression as prognostic factors in the prostatic carcinoma and

urothelial carcinoma of bladder are recommended.

Abbreviations

UC: Urothelial Carcinoma

PC: prostatic carcinoma

CK: Cytokeratin

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Conflicts of interests

The authors declare that they have no conflicts of interests.

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