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RESEARCH ARTICLE

Evaluation of pyruvate kinase M2 (PKM2) and epidermal growth factor receptor (EGFR) expression and their clinicopathologic significance in bladder urothelial carcinoma

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ABSTRACT

Background: Growing interest has been recently directed towards the roles of cancer metabolism in carcinogenesis with "Warburg effect" being the signature of cancer metabolism. Pyruvate kinase M2 (PKM2) is a crucial regulator of the Warburg effect. Epidermal growth factor receptor (EGFR) has been also involved in different metabolic pathways. **Aim**: This study aimed to investigate the immunohistochemical expression of PKM2 and EGFR in urothelial carcinoma. Furthermore, we analyzed the relation between PKM2 and EGFR with the available clinicopathologic parameters. **Materials and Methods:** PKM2 and EGFR immunostaining was performed on 70 specimens of urothelial carcinoma of the urinary bladder. **Results:** PKM2 was highly expressed in 62.9% of cases. High PKM2 expression was significantly associated with high-grade (P=0.003), muscle invasion (P=0.002), advanced T staging (P=0.001), and concomitant carcinoma in situ (P=0.013). EGFR expression was detected in 68.6% of cases. EGFR expression was significantly higher in high-grade tumors (P=0.013), muscle-invasive tumors (P<0.001), tumors with advanced T staging (P<0.001), and tumors with lympho-vascular invasion (P=0.016). There was a statistically significant positive correlation between both PKM2 and EGFR expression (r= 0.640, P <0.001). **Conclusions:** The expression of PKM2 and EGFR is positively correlated in urothelial carcinoma. High expression of PKM2 and EGFR is associated with high tumor grade, advanced T staging, and muscle invasion. Thus, they might be potentially valuable in predicting cancer prognosis in patients with urothelial carcinoma.

Keywords: Urothelial carcinoma, Tissue microarray (TMA), pyruvate kinase M2 (PKM2) and epidermal growth factor receptor (EGFR)

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INTRODUCTION

Bladder carcinoma (BCa) is the ninth most commonly diagnosed cancer worldwide with urothelial carcinoma being its most frequent histologic subtype (Siegel et al., 2021). Although early-stage tumors are usually of favorable prognosis, advanced BCa is considered one of the most lethal cancers with high morbidity and mortality (Kamat et al., 2016). Concerns about the molecular profiling of BCa have grown in order to understand their tumor biology and to develop novel therapies.

Cancer metabolism constitutes a new characteristic hallmark in cancer biology (Shan et al., 2018). Cancer cells differ fundamentally from their normal counterparts in how they utilize glucose. Normally, glycolysis increases under hypoxic conditions, but in cancers, glycolysis is enhanced even in the presence of abundant oxygen. This phenomenon is known as aerobic glycolysis or the "Warburg effect". It results in increased acidity in the tumor microenvironment which encourages tumor cell growth and proliferation (Warburg et al. 1927 and Wong et al., 2015).

Pyruvate kinase M2 (PKM2) is an essential metabolic enzyme implicated in the final rate-limiting step of glycolysis. It catalyzes the transfer of a phosphate group from phosphoenolpyruvate to adenosine diphosphate (ADP) to generate pyruvate and

adenosine triphosphate (ATP) (Yang and Lu, 2015). Besides its established role in glycolysis, PKM2 has been demonstrated to perform important roles in cell proliferation, angiogenesis and invasion (Liu et al., 2016).

The epidermal growth factor receptor (EGFR) family, including EGFR, HER-2, HER-3, and HER-4, has been implicated in tumor cell growth and differentiation (Tsai et al., 2015). EGFR is a tyrosine kinase transmembrane receptor that performs crucial functions in carcinogenesis, including cell survival, tumor metastasis, and angiogenesis (Sigismund et al., 2018).

A potential linkage between PKM2 and EGFR has been proposed (Hsu et al., 2016), but their expression and role in urothelial carcinoma have not been extensively studied. This study aimed to evaluate PKM2 and EGFR immunohistochemical expression in bladder urothelial carcinoma and to analyze the relationship between PKM2 and EGFR with the available clinicopathologic parameters.

MATERIAL AND METHODS

Study design and case selection

This cross-sectional study included 70 urothelial carcinomas specimens. Paraffin blocks were obtained from the archives of Pathology Department, Faculty of Medicine, Tanta University during the period from May 2018 to October 2020. The studied specimens were 59 samples obtained by trans-urethral resection of bladder tumor (TURBT) and 11 radical cystectomy specimens. TURBT biopsies with no identified muscularis propria were excluded. The study protocol was approved by the Research Ethical Committee at Faculty of Medicine, Tanta University.

Collection of clinicopathological data

Patients data and tumor-related characteristics (tumor size and multiplicity) were obtained from the accompanying medical and radiological reports.

Histopathologic assessment

Tumor grading was performed following WHO criteria (Humphrey et al., 2016). Depth of invasion (T staging) was assessed according to TNM American Joint Committee on Cancer/Union International Center Cancer staging system (AJCC/UICC) (Amin et al., 2017).

Tissue Microarray (TMA)

Using the TMA builder mold (CAT# TMA-001, Thermo Fisher Scientific, Runcorn, UK), recipient paraffin blocks (6x4 array) were produced. Two tissue cores from areas of interest on paraffin blocks of the studied specimens were then injected into the holes on the recipient blocks to form TMA Blocks. The selected areas were representative of tumors with good cellular preservation. Areas with necrosis, crushing artifacts, or poor cellular preservation were avoided.

Immunohistochemical staining

TMA blocks were sectioned (5 μm thick) on positively charged slides and were dried for 30 min at 37°C. The slides were placed in a Dako PT Link unit for deparaffinization and antigen retrieval. High pH EnVision™ FLEX Target Retrieval Solutions was applied, reaching 97°C for 20 min. Dako Autostainer Link 48 was used for immunohistochemistry. Briefly, slides were placed in Peroxidase-Blocking Reagent for 10 min, incubated with PKM2 rabbit polyclonal antibody (Kit No. GTX50857, GeneTex, California, USA) and EGFR mouse monoclonal antibody (Kit No. sc-373746, Santa Cruz Biotechnology, Texas, USA) for 20–30 min. Thereafter, slides were incubated with horseradish peroxidase polymer reagent for 20 min and diaminobenzidine chromogen for 10 min. Slides were then counterstained with hematoxylin.

Evaluation of PKM2 immunostaining

PKM2 expression was detected as brownish staining in the cytoplasm of tumor cells. Both the intensity and the percentage of positively stained cells were evaluated. The intensity of positivity was scored as follows: 0, negative; 1, weak; 2, moderate; and 3, strong intensity. The percentage of positive cells was scored as follow: 0%, 0; 1-24%, 1; 25-49%, 2; 50-74%,3; more than 75%, 4. The final score was obtained by multiplying the intensity and the percentage of positivity scores, which yielded a range score from 0 to 12. Score that is less than or equal to 6 indicated low expression, whereas scores

more than 6 indicated high expression (Qian et al., 2020).

Evaluation of EGFR immunostaining

EGFR immunostaining was detected as cytoplasmic and/or membranous brownish staining. Both staining intensity and percentage of positive tumor cells were considered. Staining intensity was scored as follows: 0, negative; 1, weak; 2, moderate; and 3, strong intensity. The percentage of positive cells was scored as follow: 0%, 0; 1-<10%, 1; 10-50%, 2; more than 50%, 3. Both scores were added to yield the final score from 0 to 6. Scores 0-2 indicated low expression, whereas scores 3-6 indicated high expression (Chen et al., 2020).

Statistical analysis

Statistical analysis was performed using Statistical Package for Social Science (SPSS version 23). Data were expressed as frequencies for categorical variables whereas continuous variables were expressed as mean ± SD. For comparing categorical variables, Chi-square ($\chi 2$), Fisher's exact, and Monte Carlo tests were used. Student T-test was performed for comparing two means. Spearman rank correlation was performed to evaluate the correlation between PKM2 and EGFR expression. P values less than 0.05 were considered statistically significant.

RESULTS

Clinicopathologic characteristics of the studied cases

This study included 70 urothelial carcinoma cases. The mean age of the studied cases was 63.09±8.99 years. Forty-nine cases (70%) were male and 21 cases (30%) were females. High-grade tumors constituted 70% (49 cases). Muscle invasion was evident in 60% of cases while the lymphovascular invasion was detected in 21.4% as summarized in Table 1.

Relations between PKM2 expression and clinicopathological parameters

High expression of PKM2 was detected in 44 cases (62.9%) whereas the remaining 26 cases (37.1%) displayed low PKM2 expression. The representative images are shown in Figures 1 & 2. High PKM2 expression was significantly related to high tumor grade (P=0.003), as high PKM2 expression was detected in 73.1% of high grade cases whereas two-third (66.7%) of low grade cases displayed low PKM2 expression. The relation between PKM2 expression and T staging was statistically significant (P=0.002). The two included T4 cases and 80% of T3 cases [4/5] expressed high scores of PKM2, while only one case (11.1%) of Ta cases was PKM2 high expression.

Significant associations were detected between high PKM2 expression and the presence of muscle invasion and concomitant CIS (P=0.001 and 0.013 respectively). However, the relation between PKM2 expression and lymphovascular invasion did not reach a statistically significant value (P= 0.104) as demonstrated in Table 2.

Relations between EGFR expression and clinicopathological parameters

EGFR was expressed at high scores in 68.6% (48/70) of cases whereas the remaining 22 cases (31.4%) displayed EGFR low expression. The representative images are shown in Figures 3,4. Significant associations were detected between high EGFR expression and high grade (P=0.013), T staging (P<0.001), muscle invasion (P<0.001), and lymphovascular invasion (P=0.016). High EGFR expression was detected in 76.9% of high-grade tumors, all included T3 and T4 cases, 85.7% of muscle-invasive cases, and 93.3% of cases associated with lymphovascular invasion. The relations between EGFR and clinicopathologic variables are presented in Table 3.

Correlation between PKM2 and EGFR expression

Studying the correlation between PKM2 and EGFR expression revealed a statistically significant strong positive correlation between both markers (r= 0.640, P <0.001). High PKM2 scores were associated with high EGFR scores as illustrated in Figure 5.

DISCUSSION

Aerobic glycolysis has been recognized as an important element of the malignant phenotype and a hallmark of tumor invasiveness. However, how malignant cells promote this peculiar metabolism remains uncertain. Overexpression of glycolytic genes is found to enhance tumor

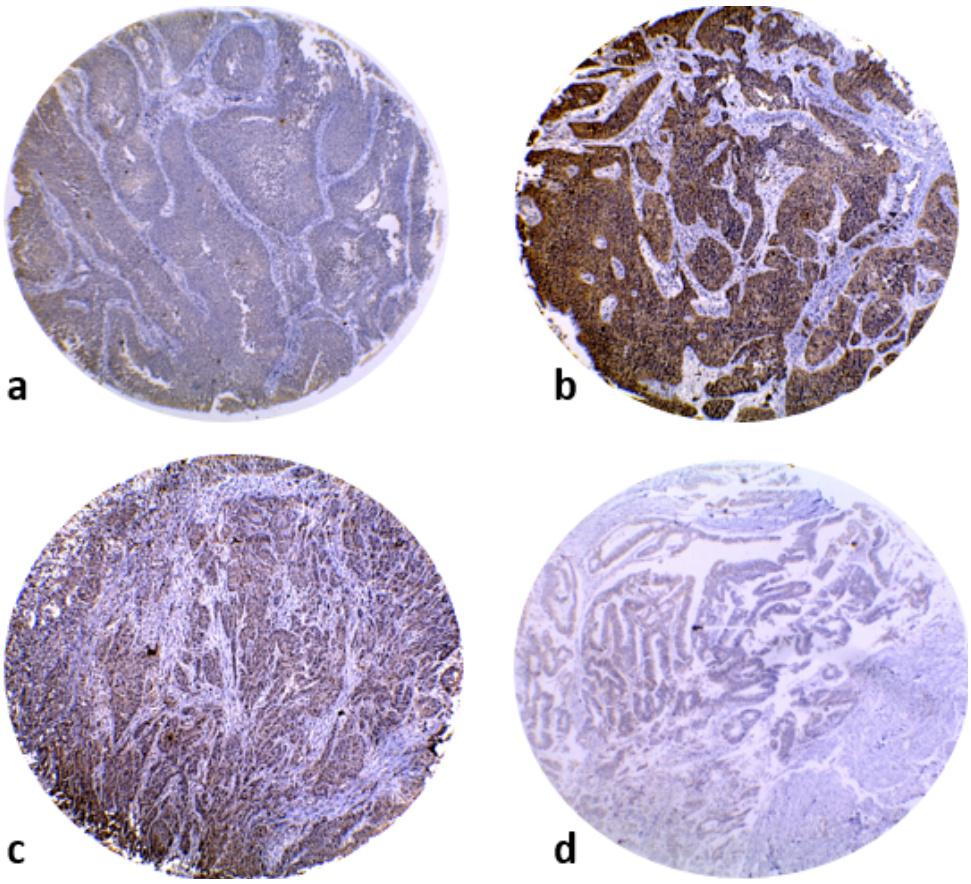

Figure 1. Tissue microarray cores stained with PKM2 (x40) a) low grade infiltrating urothelial carcinoma, b) high grade infiltrating urothelial carcinoma, c) Infiltrating urothelial carcinoma with squamous differentiation, d) Infiltrating urothelial carcinoma with glandular differentiation.

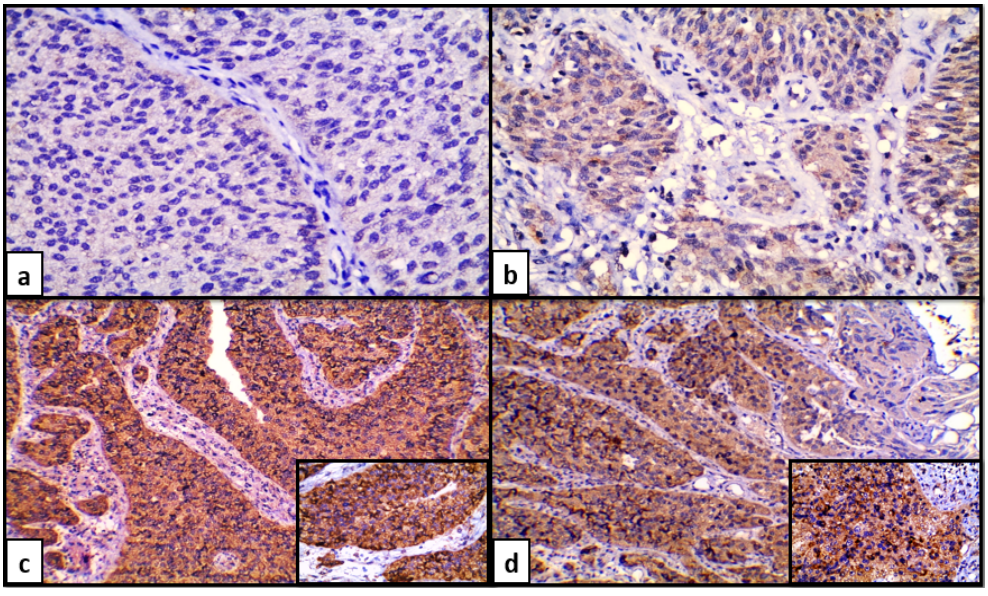

Figure 2. PKM2 immunohistochemical expression in urothelial carcinoma (UCa): a) low PKM2 cytoplasmic expression in non-invasive papillary UCa (x400), b) low PKM2 cytoplasmic expression in low grade infiltrating UCa (x400), c) high PKM2 cytoplasmic expression in high grade infiltrating UCa (x200) [inset: higher magnification x400], d) high PKM2 cytoplasmic expression in UCa with squamous differentiation (x200) [inset: higher magnification x400]

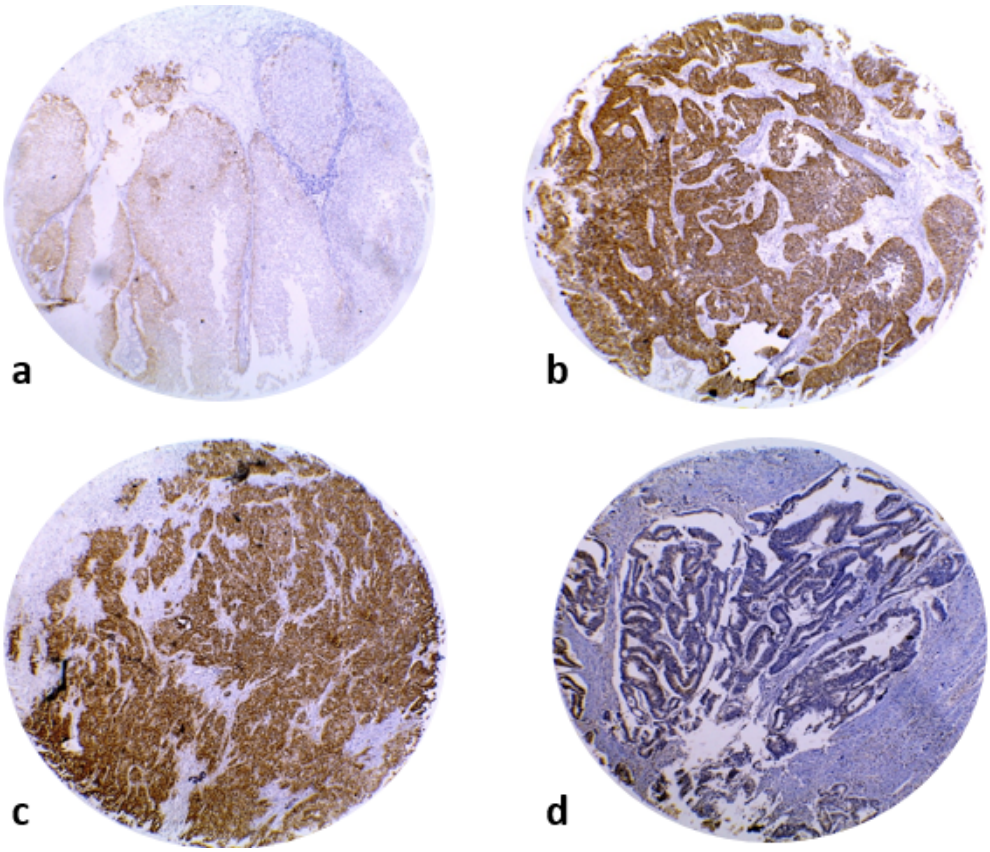

Figure 3. Tissue microarray cores stained with EGFR (x40) a) low grade infiltrating urothelial carcinoma, b) high grade infiltrating urothelial carcinoma, c) Infiltrating urothelial carcinoma with squamous differentiation, d) Infiltrating urothelial carcinoma with glandular differentiation.

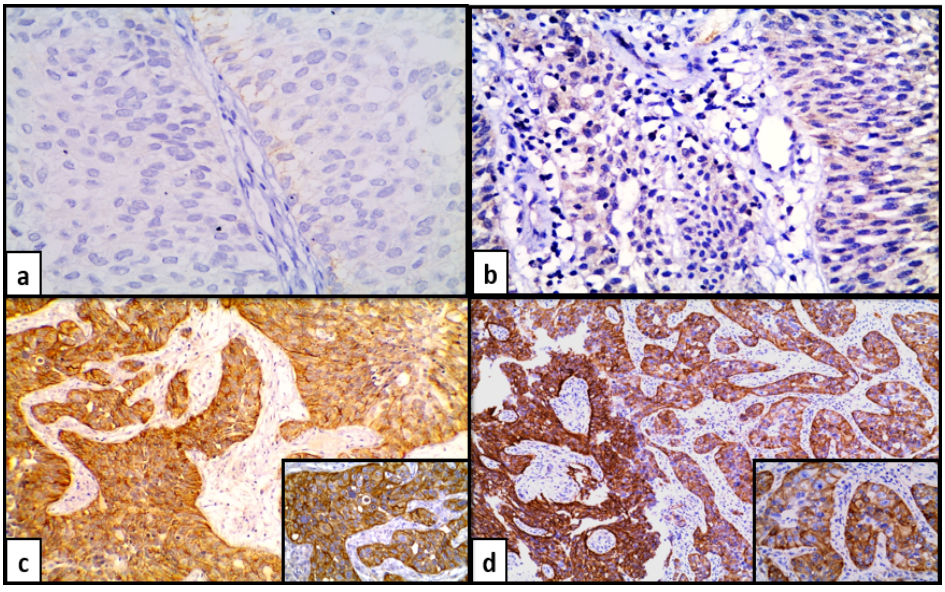

Figure 4. EGFR immunohistochemical expression in urothelial carcinoma (UCa): a) low EGFR cytoplasmic expression in non-invasive papillary UCa (x400), b) low EGFR cytoplasmic expression in low grade infiltrating UCa (x400), c) high EGFR cytoplasmic and membranous expression in high grade infiltrating UCa (x200) [inset: higher magnification x400], **d)** high EGFR cytoplasmic and membranous expression in UCa with squamous differentiation (x100) [inset: higher magnification x400]

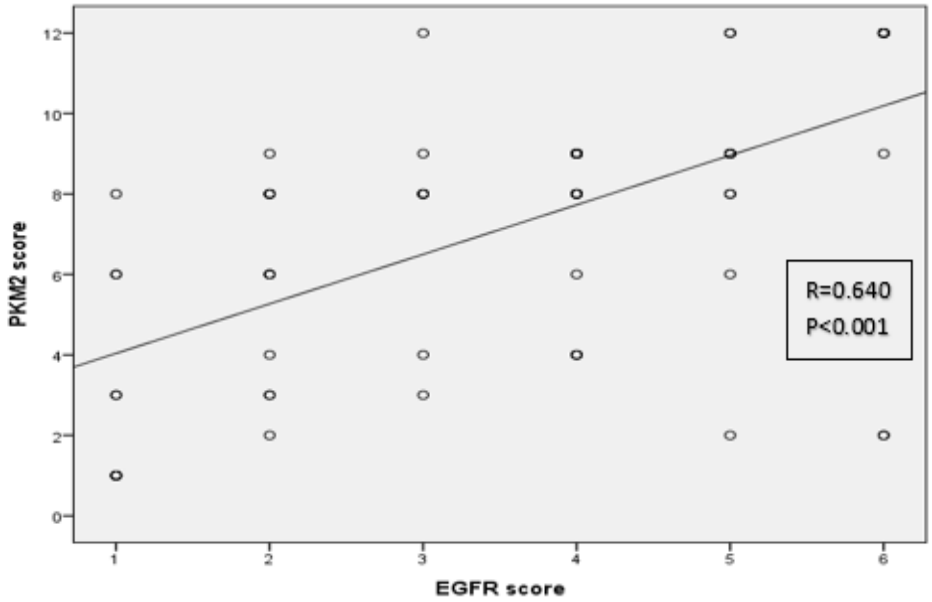

Figure 5. Scatter plot demonstrates a strong positive correlation between PKM2 and EGFR expression

aerobic glycolysis (Feng et al., 2020). PKM2 is one of the highly expressed genes and is essentially involved in carcinogenesis and tumor glucose metabolism (Liu et al., 2016). EGFR signaling has been implicated in the regulation of different metabolic pathways that are crucial for tumor growth (Sigismund et al., 2018).

Few studies have evaluated the immunohistochemical expression of PKM2 in urothelial carcinoma. In this work, PKM2 was expressed with high scores in the cytoplasm of 62.9% of cases whereas low PKM2 expression was detected in the remaining 37.1%. This rate of PKM2 expression could be related to a large number of high-grade cases, included in this study, compared to those with low grades. This result was close to those obtained by Huang et al. (2018) who reported high PKM2 expression in 54.4% of UCa cases. They demonstrated an increasing trend of PKM2 expression with increasing tumor grade. Similarly, Zhou et al. (2016) demonstrated that PKM2 is strongly expressed in urothelial carcinoma compared to adjacent normal tissue using both western blot and immunohistochemistry.

Liu et al. (2019) demonstrated that PKM2 levels in urine samples were substantially elevated in most patients with BCa. This indicates the association between the abnormal expression of PKM2 and BCa. Moreover, increased expression of PKM2 has been also reported in several cancers including gastric cancers (Lim et al., 2012), hepatocellular carcinoma (Chen et al., 2014), oral and esophageal squamous cell carcinoma (Wang et al., 2015). Collectively, these findings suggest that PKM2 is an important oncogene in the process of carcinogenesis.

The current study revealed a lack of statistically significant relations between PKM2 expression and patients' age, gender, tumor size, and tumor multiplicity. These results agreed with Huang et al. (2018). Similar results were obtained by Wang et al. (2015) in oral squamous cell carcinoma and Cui and Shi (2015) in colorectal carcinoma.

Analyzing the relation between PKM2 expression and tumor grade, the present study revealed a significant association between PKM2 expression and tumor grade. Similarly, Zhou et al. (2016) and Huang et al. (2018) demonstrated that PKM2 expression increased in low-grade UCa but became higher in high-grade invasive UCa. PKM2 plays a significant role in promoting urothelial tumor initiation and proliferation. Switching from PKM1 to PKM2 results in a decreased pyruvate kinase activity. This could slow down glycolysis and increase the accumulation of glycolytic intermediates which enhances tumorigenesis and cell division (Bayley and Devilee. 2012).

Table 1. Patient characteristics

	N (%)
Age (years) mean±SD	63.09±9.06
Gender	
Male	49 (70)
Female	21 (30)
Size (cm) mean±SD	3.74±1.86
Multiplicity	
Single	57 (81.4)
Multiple	13 (18.6)
Histologic variant	
Non-invasive papillary UCa	12 (17.1)
Infiltrating UCa	
Pure infiltrating carcinoma	38 (54.3)
UCa with divergent differentiation	20 (28.6)
Tumor grade	
Low grade	21 (30)
High grade	49 (70)
pT staging	
Ta	9 (12.9)
T1	19 (27.1)
T2	35 (50)
T3	5 (7.1)
T4	2 (2.9)
Muscle invasion	
Absent	28 (40)
Present	42 (60)
Lymphovascular invasion	
Absent	55 (78.6)
Present	15 (21.4)
Concomitant CIS	
Absent	53 (75.7)
Present	17 (24.3)
Associated schistosomiasis	
Absent	52 (74.3)
Present	18 (25.7)
PKM2	
Low expression	26 (37.1)
High expression	44 (62.9)
EGFR	
Low expression	22 (31.4)
High expression	48 (68.6)

UCa: Urothelial carcinoma, CIS: Carcinoma in situ

Also, the antagonistic effects of PKM2 to apoptosis, autophagy, and unfolded protein response could promote cell survival (Sun et al., 2011). In agreement with Huang et al. (2018), the current study described significant associations between high PKM2 expression and advanced T staging (T3 and T4) and the presence of muscle invasion. This may be attributed to increased lactate production by glycolysis leading to acidification of the extracellular environment. This enhances cell invasion and metastasis (Lim et al., 2012). In addition, PKM2 has been recognized as a key mediator of epithelial-mesenchymal transition, thus enhancing the invasiveness and metastatic potential of malignant cells (Zahra et al., 2020). So high PKM2 expression could indicate a poor prognosis. Although the present study revealed high PKM2 expression in most cases with lymphovascular invasion, this was statistically insignificant which agreed with Hu et al. (2020) in pancreatic ductal adenocarcinoma, Lin et al. (2015) in breast cancer, and Lu et al. (2018) in hepatocellular carcinoma. On the other hand, studies performed by Chen et al. (2014) and Liu et al. (2017) in hepatocellular carcinoma reported a significant relation between PKM2 expression and lymphovascular invasion.

In the present work, it was observed that PKM2 expression was significantly higher in cases with concomitant carcinoma in situ (CIS) than those without. Wang et al. (2015) suggested that PKM2 is critically implicated in carcinoma pathogenesis in oral squamous cell carcinoma. They reported that PKM2 expression was almost negative in normal epithelial but gradually became more positive in dysplasia/carcinoma in situ and invasive carcinoma. Regarding EGFR expression in the present study, it was highly expressed in 68.6% of cases. This finding was close to results obtained by Carlsson et al. (2015) and Li et al. (2018) who reported EGFR expression in 71% and 55.4% of cases respectively. On the other hand, Naik et al. (2011) and Hashmi et al. (2018) reported EGFR high expression in only 26.2% and 23% of cases respectively. This wide variation in EGFR expression may be due to different antibodies used and different scoring systems applied.

In the present work, it was observed that high EGFR expression was significantly associated with high-grade cases. These results agreed with those of Li et al. (2018) and Hashmi et al. (2018). On the other hand, a study by Khaled et al. (2009) revealed a lack of EGFR expression in correlation with high tumor grade. EGFR could promote cell proliferation, cell differentiation and could also inhibit apoptosis which is important for maintaining malignant growth. So, EGFR signaling correlates with cancer progression, metastasis, resistance to chemotherapy, and thus poor prognosis (Sasaki et al., 2013).

Table 2. Relation between PKM2 expression and clinicopathologic parameters

	Total N=70	Low PKM2 N=26 (37.1%)	High PKM2 N=44 (62.9%)	P value
Age (years) mean±SD	70	62.19±8.62	63.61±9.37	0.530
Gender				
Male	49	19 (38.8)	30 (61.2)	0.440
Female	21	7 (33.3)	14 (66.7)	
Size (cm) mean±SD	70	3.48 ±1.33	4.20±1.86	0.089
Multiplicity				
Single	57	21 (36.8)	36 (63.2)	0.576
Multiple	13	5 (38.5)	8 (61.5)	
Histologic variant				
Non-invasive papillary UCa	12	8 (66.7)	4 (33.3)	0.066
Infiltrating UCa				
Pure infiltrating carcinoma	38	12 (31.6)	26 (68.4)	
UCa with divergent differentiation	20	5 (30)	15 (70)	
Tumor grade				
Low grade	18	12 (66.7)	6 (33.3)	0.003*
High grade	52	14 (26.9)	38 (73.1)	
pT staging				
Ta	9	8 (88.9)	1 (11.1)	0.002*
T1	19	9 (47.4)	10 (52.6)	
T2	35	8 (22.9)	27 (77.1)	
T3	5	1 (20)	4 (80)	
T4	2	0 (0)	2 (100)	
Muscle invasion				
Absent	28	17 (60.7)	11 (39.3)	0.001*
Present	42	9 (21.4)	33 (78.6)	
Lymphovascular invasion				
Absent	55	23 (41.8)	32 (58.2)	0.104
Present	15	3 (20)	12 (80)	
Concomitant CIS				
Absent	57	25 (43.9)	32 (56.1)	0.013*
Present	13	1 (7.7)	12 (92.3)	
Associated schistosomiasis				
Absent	52	18 (34.6)	34 (65.4)	0.319
Present	18	8 (44.4)	10 (55.6)	

* Significant ($p<0.05$), UCa: Urothelial carcinoma, CIS: Carcinoma in situ

In agreement with Li et al. (2018) and Hashmi et al. (2018), the present study revealed significant associations between high EGFR expression and advanced T staging, muscle invasion, and lymphovascular invasion. A meta-analysis performed by Wang et al. (2014) investigated EGFR expression in esophageal SCC, they concluded that EGFR overexpression is an important predictor of T stage and vascular invasion and thus could be used as a poor prognostic indicator. This increased ability of invasion in EFGR positive tumors could be related to EGFR promoting cell motility and invasion. EGFR enhances cell migration through receptor phosphorylation and subsequent activation of downstream signaling pathways (Keller and Schmidt, 2017). Moreover, it was observed that EGFR overexpression was associated with the robust expression of matrix metallopeptidase (MMP-2 and MMP-9). These proteolytic enzymes play several key roles during angiogenesis and metastasis (Sasaki et al., 2013).

As regards the presence of concomitant CIS, the present study showed a lack of significant relation with EGFR expression. This was supported by Zangouei at al. (2020) who reported that highly expressed EGFR is typically a late event during BCa progression as a result of genomic instability.

Table 3. Relation between EGFR expression and clinicopathologic parameters

	Total N=70	Low EGFR N=22 (31.4%)	High EGFR N=48 (68.6%)	P value
Age (years) mean±SD	70	64.77±8.84	62.31±9.15	0.295
Gender				
Male	49	18 (36.7)	31 (63.3)	0.118
Female	21	2 (19)	17 (81)	
Size (cm) mean±SD				
>3 cm	70	3.14±1.46	4.02±1.97	0.065
Multiplicity				
Single	57	20 (35.1)	37 (64.9)	0.146
Multiple	13	2 (15.4)	11 (84.6)	
Histologic variant				
Non-invasive papillary UCa	12	7 (58.33)	5 (41.76)	0.069
Infiltrating UCa				
Pure infiltrating carcinoma	38	11 (28.9)	27 (71.1)	
UCa with divergent differentiation	20	4 (20)	16 (80)	
Tumor grade				
Low grade	18	10 (55.6)	8 (44.4)	0.013*
High grade	52	12 (23.1)	40 (76.9)	
pT staging				
Ta	9	8 (88.9)	1 (11.1)	<0.001*
T1	19	8 (42.1)	11 (57.9)	
T2	35	6 (17.1)	29 (82.9)	
T3	5	0 (0)	5 (100)	
T4	2	0 (0)	2 (100)	
Muscle invasion				
Absent	28	16 (57.1)	12 (42.9)	<0.001*
Present	42	6 (14.3)	36 (85.7)	
Lymphovascular invasion				
Absent	55	21 (38.2)	34 (61.8)	0.016*
Present	15	1 (6.7)	14 (93.3)	
Concomitant CIS				
Absent	57	19 (33.3)	38 (66.67)	0.472
Present	13	3 (23.08)	10 (76.92)	
Associated schistosomiasis				
Absent	52	16 (30.8)	36 (69.2)	0.529
Present	18	6 (33.3)	12 (66.7)	

* Significant ($p<0.05$), UCa: Urothelial carcinoma, CIS: Carcinoma in situ

Although Badawy et al. (2017) demonstrated that Schistosoma infection with BCa is associated with increased EGFR expression, this study reported a lack of significant association between EGFR expression and Schistosoma infection. This conflict may be explained that nearly 75% of cases within the present study were not associated with Schistosoma infection.

On analyzing the correlation between PKM2 and EGFR expression, the present study was able to detect a strong positive significant correlation between PKM2 and EGFR expression as high PKM2 scores were associated with high EGFR scores. This agreed with Yang et al. (2016) who stated that PKM2 positivity was correlated with mutant EGFR expression and that PKM2 stabilized mutant EGFR protein by direct interaction in lung cancer cells. Moreover, PKM2 inhibition resulted in markedly decreased mutant EGFR expression.

Similarly, Chen et al. (2020) found that knockdown of either EGFR signaling or PKM2 in nasopharyngeal carcinoma can inhibit tumor cell invasiveness and metastatic potential. Hsu et al. (2016) demonstrated that PKM2 enhances EGFR phosphorylation and stimulates the EGFR downstream signaling in triple-negative breast cancer cells. EGFR promoted glycolysis and tumorigenesis require protein kinase C and nuclear factor κ enhancer binding protein

(NFκB) and both depend on PKM2 upregulation (Dong et al., 2016).

On the other hand, Lim et al. (2016) suggested that EGFR plays a crucial function in the upregulation of PKM2 and subsequently enhancing tumorigenesis of cells. EGFR signals stimulate the switch from glycolytically active to inactive PKM2, resulting in a slowdown of aerobic glycolysis with an accumulation of metabolic intermediates, promotion of tumor growth.

Taken together, PKM2 expression seems to be linked to EGFR expression, but the precise mechanisms that regulate this association need to be elucidated in future studies.

CONCLUSION

The expression of PKM2 and EGFR is positively correlated in urothelial carcinoma. High expression of PKM2 and EGFR is associated with high tumor grade, advanced T staging, and muscle invasion. Thus, they might be potentially valuable in predicting cancer prognosis in patients with urothelial carcinoma.

CONFLICTS OF INTEREST

All authors declare no conflicts of interest.

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