Predictive Value of PKM2 Expression in Advanced Non-Small Cell Lung Cancer Patients (NSCLC) Treated with First line Platinum-based Chemotherapy

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Abstract: Background: The aim of the study was, to assess the expression of pyruvate kinase isoform M2 (PKM2) in advanced NSCLC patients treated with first line platinum-based chemotherapy and evaluate its predictive value on both progression free survival (PFS) and overall survival (OS). **Methods:** 72 cases with histologically confirmed stage IIIB and IV NSCLC who were treated with front-line platinum-based chemotherapy. Thirty two NSCLC patients were treated with first line non-platinum-based doublets were enrolled in this study (as control), Immunohistochemical staining for PKM2 was evaluated. **Results:** in Platinum group the median OS was 7 vs 19 months; P < 0.001 for those patients with high compared to those with low PKM2 expression respectively and the median PFS was 5 vs 9 months; P < 0.001 for those patient with high compared to those with low PKM2 expression respectively. In control group there was no significant difference between high and low PKM2 expression as regard median OS (9 vs 10 months; P =< 0.451) and median PFS (7 vs 8 months; P = 0.638). The multivariate analysis revealed that high PKM2 expression was an independent predictive factor for shorter PFS and decreased OS. **Conclusions:** Our study showed that PKM2 expression is a predictive biomarker of platinum treatment outcome in advanced NSCLC patients.

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Keywords: PKM2 expression, advanced non-small cell lung cancer patients (NSCLC), first line platinum-based chemotherapy

1. Introduction

About 220,000 new cases of lung cancer are expected to be diagnosed in 2015 while overall 58,000 people are expected to die from lung cancer in the same year.[1]

Patients diagnosed with non-small cell lung cancer (NSCLC) represent more than 80 % of those with lung cancers[2], and about fifty percent of those patients present with advanced disease (stage III or IV).[3]

Systemic platinum-based chemotherapy is the backbone for the treatment of advanced NSCLC as it improves symptoms, quality of life and survival compared to best supportive care.[4]

Recently there were evidences led to the detection of molecular markers related with resistance to platinum agents one of them is PKM2 and its role in this aspect is under extensive investigation.[5]

Consumption of glucose is more in most tumors in comparison to normal tissue with increased lactate production in the presence of oxygen, which is known as aerobic glycolysis, of which pyruvate kinase (PK) is considered as the main regulator. PK is a key rate-limiting enzyme that catalyzes the final step of glycolysis, converting phosphoenol-pyruvate to pyruvate while phosphorylating adenosine diphosphate (ADP) to adenosine triphosphate (ATP).[6] Also, this enzyme is involved in a variety of pathways, protein–protein interactions, and nuclear transport indicates its importance to perform multiple non-glycolytic functions with many effects, although multiple roles of this enzyme are as yet not fully detected.[7]

Aim of the Work

Assess the expression of pyruvate kinase isoform M2 (PKM2) in advanced NSCLC patients treated with first line platinum-based chemotherapy and evaluate its predictive value on both progression free survival (PFS) and overall survival (OS).

2. Patients and Methods

This prospective study was carried out on seventy two cases with histologically confirmed stage IIIB and IV NSCLC who were treated with front-line platinum-based chemotherapy. Thirty two NSCLC patients were treated with front-line non-platinum-based doublets were enrolled in this study (as control).

All patients were treated and followed up at Clinical Oncology Departments, Tanta university hospital during the period from December 2013 to April 2016. Written, informed consent was obtained from each patient before enrollment into the study.

Patients had to have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2 [8]; with adequate organ functions and baseline imaging studies were done.

Histopahological examination

A total of 72 specimens of formalin-fixed, paraffin-embedded lung masses were analyzed. Cases were classified histologically after staining with H & E staining according to the recent WHO classification and grading.[9]

The study included 18 cases of squamous cell carcinoma, 43 cases of adenocarcinoma, 11 cases of large cell carcinoma. The cases were graded into grade 1, 2 and 3. Then clinic-pathologic stage was determined according to the TNM classification systemof the International Union against Cancer.[10]

Immunohistochemical staining for PKM2 and its evaluation

For the study, 4-µm-thick serial sections of formalin fixed, paraffin-embedded tissue were cut and mounted on positively charged glass slides. After incubation at 60°C overnight and deparaffinization, the tissue sections subjected heat retrieval for 20 minutes with Tris-EDTA buffer (Thermo Fisher Scientific, Waltham, MA). Polyclonal rabbit anti-human PKM2 antibody (PKM2 (H-60), 1:50, Santa Cruz Biotechnology, Santa Cruz, CA). The standard avidin-biotin peroxidase complex (ABC) technique was performed using the LabVision Secondary Detection Kit (Ultra Vision Detection System Anti-polyvalent, HRP). The color was visualized by incubation with chromogen 3,3'diaminobenzidine for 5 minutes. The slides were then counterstained with Mayer hematoxylin and cover slipped with Permount (StatLab, McKinney, TX). Negative controls were set for each test without the primary antibodies. Positive control was done using the adjacent normal lung tissue in 10 cases. Results were expressed semi quantitatively. Saturation and intensity of immune-stained cells was evaluated over 8 visual fields at a power of ×400 under a light microscope (Olympus Optical, Tokyo, Japan). Total staining of PKM2 was scored as the product of the staining intensity (on a scale of 0–3: negative=0, weak=1, moderate=2, strong=3) × the percentage of cells stained (positively recorded on an ordered categorical scale: 0 =zero, 1=1–25%, 2 =26–50%, 3=51–100%), which resulted in a scale of 0–9. In correlation analysis, the survival data of patients were classified as low (0–4) and high (>4) expression of PKM2.[11]

Statistical Considerations

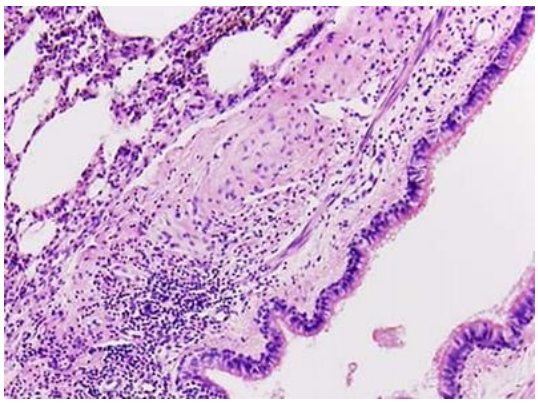
The data were analyzed using SPSS 21.0 software package. The correlation of PKM2 expression with different clinic-pathologic characteristics was analyzed with chisquare test. The Kaplan–Meier method and Log-rank test were used to analyze the correlation of patient survival with PKM2 expression. Overall-survival was defined as the time from diagnosis to the date of death from any cause or last follow-up. Progression-free survival (PFS), which was defined as the time from study to documented disease progression or death. A significance level of $P < 0.05$ was used.

3. Results

Histopathological and immunohistochemical results:

The study included 17 squamous cell carcinoma, 43 cases of adenocarcinoma, 12 large cell carcinoma with variable grades ranged from 1-3 and variable stage (fig 2a-7a)

a



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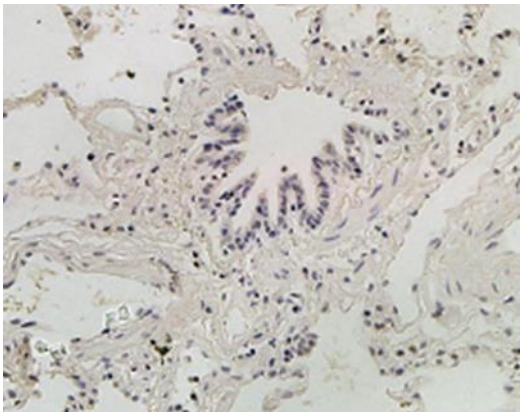


Fig 1. Normal lung tissue that showsnormal lining epithelial cells in the bronchioles and the alveoli **(a)** Showed weak expression of PKM2 in the epithelial cells **(b)** [x200]

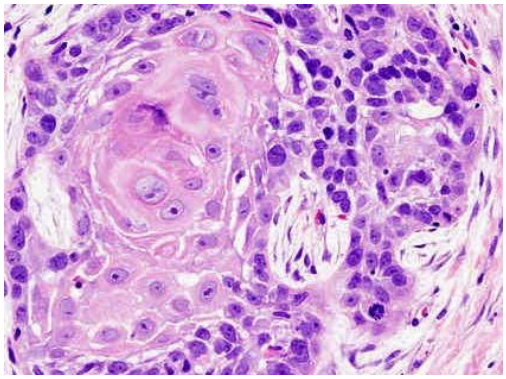
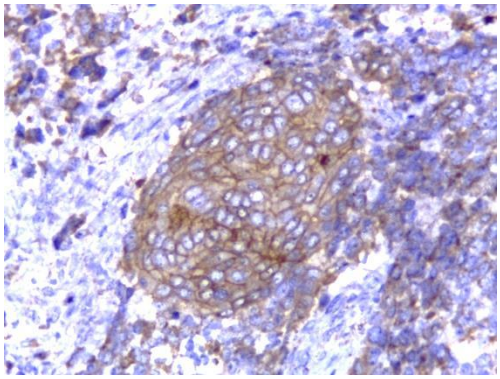
a  **b** 

Fig 2. A case of squamous cell carcinoma grade II **(a)** Showed low expression of PKM2 **(b)** (score 4). [x400]

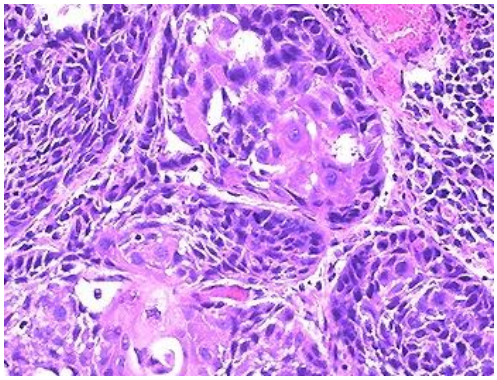
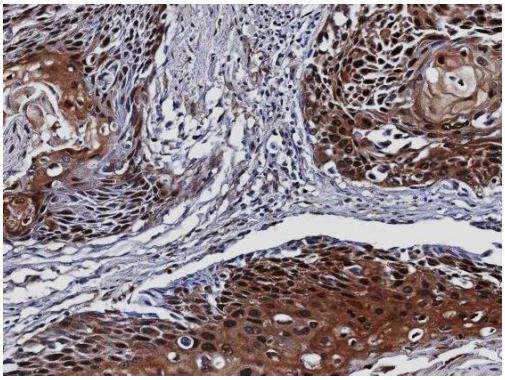
a  **b** 

Fig 3. a case of squamous cell carcinoma grade II **(a)** Showedhigh expression of PKM2 **(b)** (score 6). [x400]

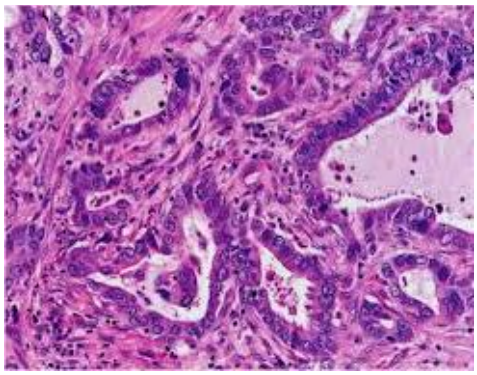
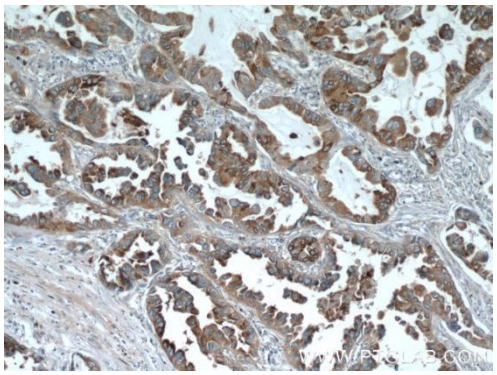
a  **b** 

Fig 4. a case of adenocarcinoma grade II **(a)** Showedlow expression of PKM2 **(b)** (score 4). [x400]

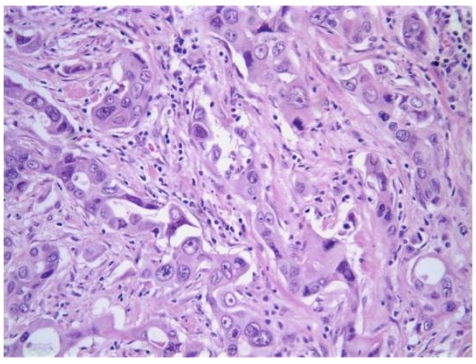
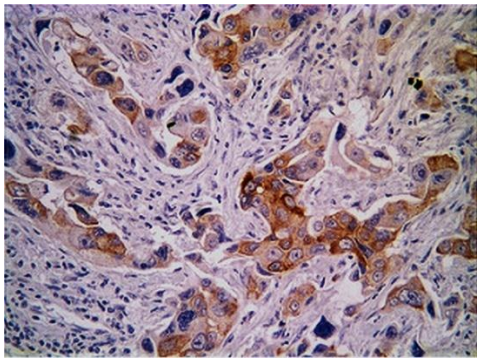
a  **b** 

Fig 5. a case of adenocarcinoma grade III **(a)** Showedhigh expression of PKM2 **(b)** (score 7). [x400]

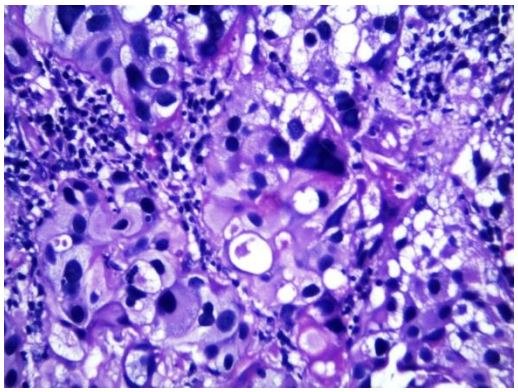
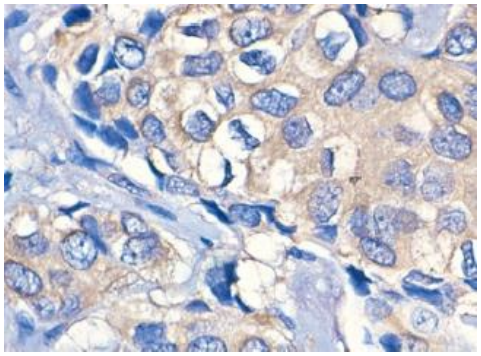
a  B 

Fig 6. a case of large cell carcinoma grade III **(a)** Showedlow expression of PKM2**(b)** (score 3). [x400]

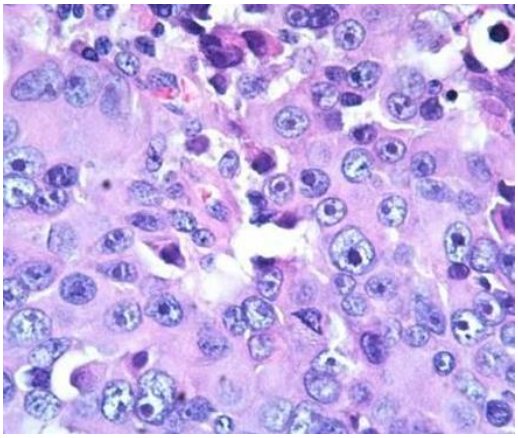
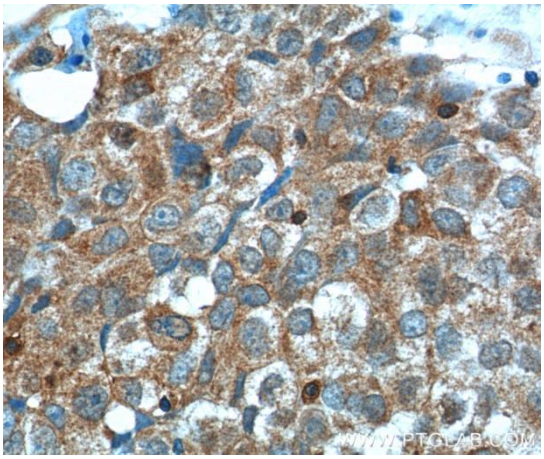
a  b 

Fig 7. a case of large cell carcinoma grade III **(a)** Showed high expression of PKM2**(b)** (score 8). [x400]

Regarding PKM2 expression, in normal lung tissue which acts as a control, all the 10 cases showed low expression (Fig 1) while in the studied tumorous cases, it was showed variable expression values (Fig 2-7). In squamous cell carcinoma cases, there were no significant correlation between the grade of the tumor and PKM2 expression while this correlation was statistically significant in adenocarcinoma and large cell carcinoma as its expression increase in high grade tumors. Table (1)

Table 1. Correlation between the grade and PKM2 expression in the studied cases

Studied cases		PKM2 expression		*P*
		Low	High	
Squamous cell carcinoma n=17	Grade 1	3	0	0.12
	Grade 2	5	2	
	Grade 3	5	2	
Adenocarcinoma n=43	Grade 1	1	3	0.03*
	Grade 2	17	4	
	Grade 3	8	10	
Large cell carcinoma n=12	Grade 1	0	0	0.001*
	Grade 2	1	1	
	Grade 3	1	9	

Abbreviations: **n** denotes patient number ***P** < 0.05**.**

Patient and tumors characteristics:

Patient and tumors characteristics are summarized in Table 2. The mean age was 63.5 and 62.7 years for the Platinum group and the control group respectively. Most patients in both groups were males received their treatment in Clinical Oncology department, Tanta University Hospital. In both groups more than two third of the lesions were non- squamous and the rest were squamous cell carcinoma. Tumor grades 3 represented in 48.6% and 46.9% of the patients in Platinum group and the control group respectively. Performance status 0-1 by ECOG scale represented in approximately half of the patients in both groups. PKM2 expression was low in one half of the patients and high in the other half in both groups.

Table 2. Patient and tumors characteristics

	Platinum group (N = 72)		Control group (N = 32)	
Characteristics	**No.**	**%**	**No.**	**%**
Mean age, years	**63.5**		**62.7**	
SD	**10.50**		**11.11**	
Range	**34-74**		**39-74**	
Gender				
Male	**56**	**77.8**	**20**	**62.5**
Female	**16**	**22.2**	**12**	**37.5**
ECOG Performance status				
0-1	**34**	**47.2**	**17**	**53.1**
2	**38**	**52.8**	**15**	**46.9**
Histological types				
Squamous cell carcinoma	**17**	**23.6**	**5**	**15.6**
Non- Squamous cell carcinoma	**55**	**76.4**	**27**	**84.4**
Tumor grade				
G1-2	**37**	**51.4**	**17**	**53.1**
G3	**35**	**48.6**	**15**	**46.9**
Treatment regimens				
Platinum + docetaxel	**54**	**75**	**NA**	
Platinum + gemcitabine	**11**	**15.3**	**NA**	
Platinum + docetaxel + Avastin	**7**	**9.7**	**NA**	
Docetaxel + gemcitabine	**NA**		**32**	**100**
Post-progression treatment	**52**	**72.2**	**25**	**78.1**
PKM2expression				
Low	**36**	**50**	**16**	**50**
High	**36**	**50**	**16**	**50**

Abbreviations: **SD** = Standard Deviation; **NA** = not applicable.

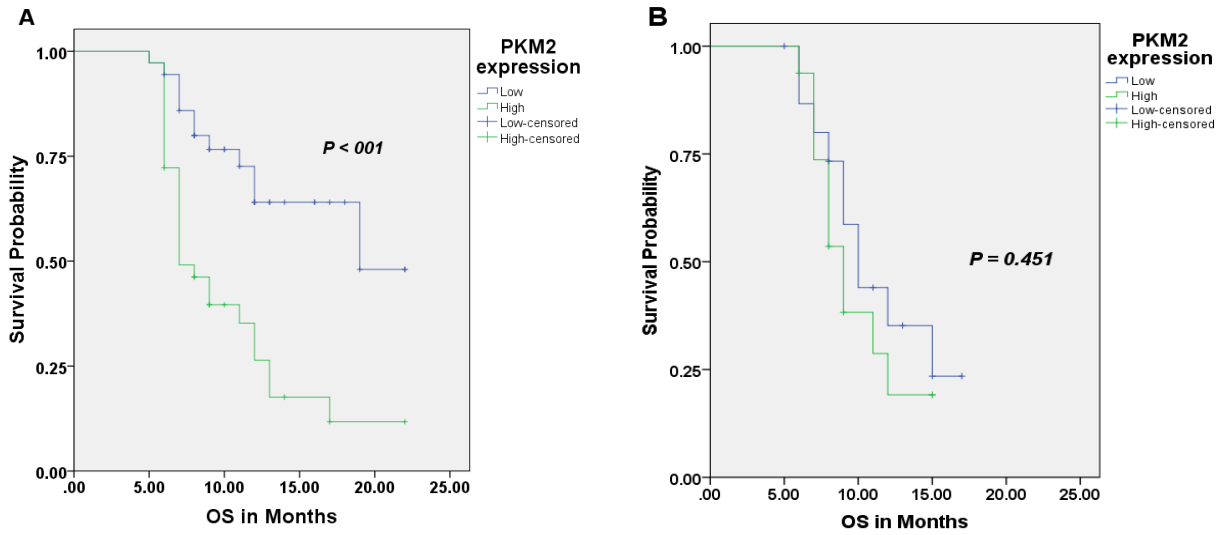


Figure 8. (A) Overall survival curve for PKM2 expression in Platinum group. **(B)** Overall survival curve for PKM2 expression in control group.

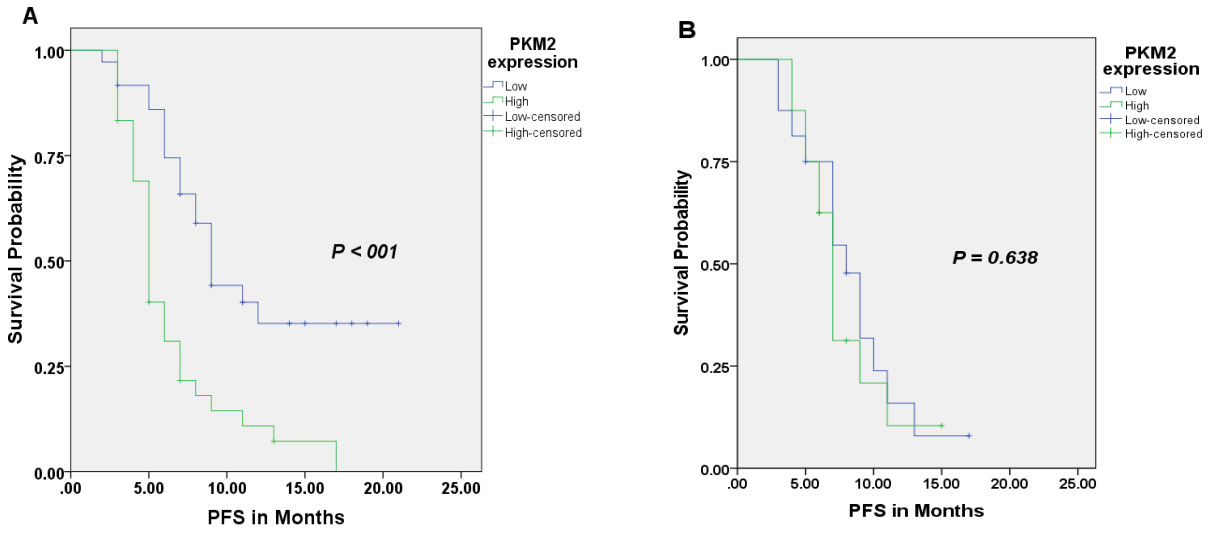


Figure 9. (A) Progression free survival for PKM2 expression in Platinum group. **(B)** Progression free survival for PKM2 expression in control group.

Survival analysis:

Kaplan-Meier analyzes were also conducted the overall survival and progression free survival curves for PKM2 expression in both groups and presented in Figure 8 and 9 respectively where in Platinum group the median OS was 7*vs*19 months; *P*< 0.001 for those patient with high compared to those with low expression respectively and the median PFS was 5 *vs* 9 months; *P* < 0.001 for those patient with high PKM2 expression compared to those with low expression respectively. In control group there was no significant difference between high and low PKM2 expression as regard median OS (9 *vs* 10 months; *P* =<0.451) and median PFS (7 *vs* 8 months; *P* = 0.638).

The variables found to be strongly significant (*P*< 0.05) with overall survival and progression free survival in the univariate Cox proportional hazards regression analyzes included age, performance status and PKM2 expression, while other was no survival significant relationship with sex, tumor grades and histological types as showed in tables 3 and 4 respectively.

Table 3. Overall survival and Univariate Hazard Ratios From Cox Proportional Hazards

Variable	Overall survival	HR	95% CI	*P*
Age				
>70 *vs*	22.5%	2.80	1.31 to 5.98	0.008*
≤70	42.8%			
Sex				
Female *vs*	48.0%	0.63	0.28 to 1.44	0.272
Male	24.6%			
Performance status				
2 *vs*	0.00%	4.11	1.98 to 8.53	< 0.001*
0-1	65.3%			
Histological types				
Non- Squamous *vs*	38.1%	0.96	0.47 to 1.94	0.901
Squamous	24.4%			
Tumor grade				
G3 *vs*	27.6%	1.90	1.00 to 3.64	0.052
G1-2	34.4%			
PKM2 expression				
High *vs*	11.7%	3.29	1.65 to 6.54	0.001*
Low	48.0%			

Abbreviations: CI denotes confidence interval; HR denotes hazard ratio
***P* < 0.05.**

Table 4. Progression free survival and Univariate Hazard Ratios From Cox Proportional Hazards				
Variable	**Progression free survival**	**HR**	**95% CI**	***P***
Age				
>70 *vs*	06.5%	2.42	1.30 to 4.51	0.005*
≤70	36.2%			
Sex				
Female *vs*	21.9%	0.89	0.47 to 1.70	0.729
Male	15.2%			
Performance status				
2 *vs*	00.0%	3.09	1.70 to 5.62	< 0.001*
0-1	33.8%			
Histological types				
Non- Squamous *vs*	23.1%	0.92	0.50 to 1.70	0.789
Squamous	08.3%			
Tumor grade				
G3 *vs*	17.5%	1.50	0.87 to 2.61	0.148
G1-2	18.5%			
PKM2 expression				
High *vs*	00.0%	2.91	1.64 to 5.15	< 0.001*
Low	35.2%			
Abbreviations: CI denotes confidence interval; HR denotes hazard ratio ***$P < 0.05$.**				

Table 5. Multivariate Hazard Ratios From Cox Proportional Hazards for OS			
Variable	Hazard Ratio	95% CI	*P*
Age (>70 *vs* **≤70 years)**	1.80	0.82 to 3.97	0.146
Performance status (2 *vs* **0-1)**	2.77	1.27 to 6.06	0.011*
PKM2 expression (high *vs* **low)**	2.24	1.09 to 4.58	0.027*
Abbreviations: CI denotes confidence interval; HR denotes hazard ratio. ***$P < 0.05$.**			

Table 6. Multivariate Hazard Ratios From Cox Proportional Hazards for PFS			
Variable	Hazard Ratio	95% CI	*P*
Age (>70 *vs* **≤70 years)**	1.73	0.90 to 3.34	0.103
Performance status (2 *vs* **0-1)**	2.06	1.07 to 3.96	0.031*
PKM2 expression (high *vs* **low)**	2.14	1.17 to 3.91	0.014*
Abbreviations: CI denotes confidence interval; HR denotes hazard ratio. ***$P < 0.05$.**			

Table 5 summarizes multivariate hazard ratios and 95% CIs for OS for the variables that were significant in univariate analysis where performance status (hazard ratio [HR]: 2.77, 95% CI: 1.27- 6.06; *P*= 0.011) and PKM2 expression (hazard ratio [HR]: 2.24, 95% CI: 1.09- 4.58; *P*= 0.027) were significant and they represented as an independent predictive factors for OS.

Table 6 summarizes multivariate hazard ratios and 95% CIs for PFS for the variables that were significant in univariate analysis where performance status (hazard ratio [HR]: 2.06, 95% CI: 1.07- 3.96; *P*= 0.031) and PKM2 expression (hazard ratio [HR]: 2.24, 95% CI: 1.17- 3.91; *P*= 0.014) were significant and they represented as an independent predictive factors for OS.

4. Discussion

In general, systemic therapy is recommended for the patients with metastatic disease.[12] Patients with stage IV disease with good performance status benefit from chemotherapy, usually with a platinum-based regimen.[13,14]

PKM2 is an isoenzyme of the glycolytic enzyme pyruvate kinase and is expressed in some differentiated tissues, such aslung,[15]and in all cells with a high rate of nucleic acid synthesis, such as normal proliferating cells, embryonic cells, and especially tumor cells.[16]

Pyruvate kinase catalyzes the last step within glycolysis and it is responsible for net ATP production within the glycolytic sequence. In contrast to mitochondrial respiration, energy regeneration by pyruvate kinase is independent from oxygen supply

and allows survival of the organs under hypoxic conditions found in solid tumors.[17], although multiple role of this protein is as yet not fully recognized.[7] However, a functional role in angiogenesis the so-called process of blood vessel formation by interaction and regulation of Jmjd8 has been shown.[18]

PKM2 exists *in vivo* in two forms, dimers and tetramers. In the normal cells, PKM2 mainly exists in the tetramer form while in the cancer cells it mainly exists as dimers.[19]

When PKM2 is in the less active dimeric form, which is the case in the tumor cells, all glycolytic intermediates above pyruvate kinase accumulate and are involved into synthetic processes, which branch off from glycolytic intermediates such as nucleic acids, phospholipids, and amino acids which are important cell building-blocks, and greatly needed by highly proliferating cells, such as tumor cells, [20]and PKM2 was found to be required for the proliferation and survival of some tumors in vivo.[21]

In our study we assessed the expression of PKM2 in advanced NSCLC patients who were treated with front-line platinum-based chemotherapy and analyze its predictive value on both progression free and overall survival and we observed that patients with low levels of PKM2 expression had significantly better PFS and OS ($P< 0.001$) than patients with high levels of PKM2 expression. Unlike the results in the platinum group, in the control group of 32 patients, who received regimens not containing platinum, there was no significant difference between those patients with low and high levels of PKM2 expression as regard PFS (P=0.638) and OS (P=0.451). Moreover multivariate analysis revealed that high PKM2 expression was an independent predictive factor for shorter PFS and decreased OS in platinum group.

Consistent with our findings, Papadaki et al reported that PKM2 expression was predictive for response to platinum-based regimens, with higher response rate in patients with low PKM2 levels while high PKM2 expression predicted for shorter survival.[4]Also, Karachaliou et al demonstrated that limited-stage small cell lung cancer patients with high expression of *PKM2* had shorter median PFS (P=0.046) and OS (P=0.026).[5]

In contrast to our finding, Yoo et al reported that PKM2 was identified as a protein that showing lower expression in cisplatin-resistant cells in human gastric carcinoma.[22]

In correlation to our finding, Multiple studies have shown that interfering with PKM2 expression with shRNA and miRNA can both lead to cell apoptosis, reduced metabolic activity, and decreased tumorigenicity.[23,24] Other studies have shown that interfering PKM2 expression with shRNA can increase the sensitivity of the cancer cells to docetaxel and cisplatin, thus promoting cell apoptosis and decreasing tumorigenicity.[25,26] Also Benjamin et al study concluded that PKM2 knockdown impaired glioma cell metabolism, with decreased glutathione and ATP levels and accelerated activation of AMP-activated protein kinase. Since PKM2 is expressed in glioma cells and is important for their survival, but is lacking in the normal brain, its manipulation may serve as a brain-sparing therapy for glioblastoma.[24] From this study inhibition of PKM2 may decrease glutathione level and one of the mechanisms explain resistance of tumor cells to cisplatin is increased glutathione.[27] So, PKM2 may play indirect role in tumor cells resistance to platinum agents.[28]

Steven et al study the role of PKM2 activator in treatment of cancer as pyruvate kinase converts phosphoenol-pyruvate to pyruvate, catalyzing the rate-limiting step of glycolysis. The M1 isoenzyme of pyruvate kinase (PKM1) is found in adult tissues; whereas, PKM2 is another variant found in embryonic and cancer cells. PKM2 expressed in malignant cells is a result of the tumor microenvironment and it is responsible for maintaining a glycolytic phenotype. PKM2 has other non-metabolic functions in malignant cells, including transcriptional co-activation and protein kinase activity. PKM2 activators have antitumor effects by inducing tetramerization of two PKM2 dimers causing PKM2 to function like PKM1. PKM2 activators have therapeutic potential in the treatment of cancer and other metabolic diseases.[29]

Conclusion

Our study showed that PKM2 expression is a predictive biomarker of platinum sensitivity in advanced NSCLC patients treated with platinum-based chemotherapy.

Compliance with Ethical Standards

Conflict of Interest

The author Mohamed F. Sheta declared that he has no conflict of interest. The author Omnia Abd –El-Fattah declared that she has no conflict of interest. The author Hanan A. Alshenawy declared that she has no conflict of interest.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent:

Informed consent was obtained from all individual participants included in the study.

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