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# Role of Serum and Bile Level of Pyruvate Kinase in Diagnosis of Malignant Biliary Obstruction: Article Review

**Mahmoud Abdel Samad Abdel Azeim [1]; Walid Ahmady Abd El-Dayem [1]; Talaat Fathy Aly [1]; Mohamed Ibrahim Magdy [1]; Atef Gouda Hussien [2]**

**[1] Tropical Medicine Department, Faculty of Medicine, Zagazig University, Egypt.**
**[2] Biochemistry and Molecular Biology, Faculty of Medicine, Zagazig University, Egypt.**

Corresponding author: **Mahmoud A. Abdel Azeim**, Email: maamohamed@medicine.zu.edu.eg

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## Abstract

Malignant biliary obstruction (MBO) presents both a diagnostic and therapeutic challenge. It is a common problem, with as many of 70% of pancreatic cancer patients presenting with obstruction upon diagnosis. Commonly, MBO appears as painless jaundice with anorexia and weight loss as the initial sign of disease such as in the classic presentation in pancreatic ductal adenocarcinoma or may occur during progression of malignancy. The presence of malignant biliary obstruction is typically associated with a poor 5-year survival rate of approximately 5%**.** Relief of the obstruction may lead to improved quality of life and extended survival. The determination of life expectancy is difficult and primarily dependent on the following factors including clinical status, metastatic disease, extent of local invasion, and use of palliative chemotherapy. Evidence supports a potential role of pyruvate kinase PKM2 in tumorigenesis. As an embryonic isoform, PKM2 is reactivated in tumors and overexpressed in multiple cancer types. Therefore, the aim of the present study was to review the significant role of pyruvate kinase in the diagnosis of malignant biliary obstruction.

**Keywords:** Malignant Biliary Obstruction; Diagnosis ; Pyruvate Kinase

## Introduction

Biliary tree obstruction and consequent jaundice occur in 70–90% of these patients and have important consequences mainly for the patient's quality of life, morbidity, and overall mortality. Options for palliative therapy of biliary tree obstruction include surgical bypass, percutaneous external drainage/stenting, and endoscopic stenting. For patients with resectable tumors, ongoing debate remains on whether preoperative drainage is necessary **(1,2)**.

Although the diagnosis can be achieved without tissue biopsy, it is important to have histological confirmation. Tissue can be acquired through interventional radiology (ultrasound/computed tomography-guided puncture) or through endoscopic procedures, such as Endoscopic Retrograde Cholangiopancreatography (ERCP) and Endoscopic Ultrasound-Guided Fine-Needle Aspiration (EUS-FNA), although the former can be associated with seeding in the needle tract **(3,4)**.

Early diagnosis is difficult to establish as biliary obstruction can be the first presentation of the underlying disease, which can already be at an advanced stage. Consequently, the majority of patients (70%) with malignant distal biliary obstruction are unresectable at the time of diagnosis **(5**). So, we review the role of pyruvate kinase as a diagnostic marker for MBO.

Pyruvate kinase (PK), as one of the key enzymes of glycolysis, acts on its substrate phosphoenolpyruvate (PEP) to form pyruvate, and pyruvate kinase (PK) has four different subtypes: L, R, M1, M2 **(6)**. PKL isoforms are mainly found in liver, kidney and red blood cells; while PKR is mainly expressed in red blood cells, biological activity is not clear; PKM1 is distributed in myocardium, skeletal muscle and brain tissue; PKM2 is the embryonic isoform and is expressed in cancer. PKM2 is also expressed in normal proliferating cells, such as lymphocytes and the cells of the intestinal epithelium **(7)**.

Pyruvate kinase can be present as a tetrameric or a dimeric form. The tetrameric structure is an active form with high binding affinity to PEP, while the dimeric form is less active with low binding affinity to PEP. The single exon difference between PKM1 and PKM2 leads to important function distinctions. PKM1 constitutively oligomerizes to a tetramer under physiological conditions, while PKM2 may be present as dimer or tetramer depending on the corresponding regulators **(8)**.

Fructose-1,6-bisphosphate (FBP), a glycolytic intermediate, preferentially binds to PKM2, but not PKM1, and consequently increases the affinity of PKM2 to PEP. In addition to FBP, many other metabolites, amino acids, and small molecules are involved in the regulation of PKM2 activity. The binding of small molecule PKM2 activators to PKM2 promotes tetramer formation, constitutively activating PKM2 and suppressing tumorigenesis. Post-translational modification of PKM2 such as through phosphorylation, acetylation, or oxidation facilitates the low activity of dimeric PKM2 **(9)**.

The alteration in PKM2 activity is related to cellular proliferation and tumor growth. The deletion of PKM2 in normal cells results in the expression of PKM1 and induces proliferation arrest by impairing nucleotide production and subsequent DNA synthesis **(10)**. A switch from PKM1 to PKM2 has been detected in various cancers, and a reverse isoform switch from PKM2 to PKM1 has been found to inhibit aerobic glycolysis and reduce tumorigenesis in a nude mouse xenograft model **(11)**.

**Roles of PKM2 in cancer types**

PKM2 is involved in many pathological processes of numerous tumor types such as gastrointestinal cancer, hepatocellular carcinoma and lung cancer. PKM2 can promote tumor growth, metastasis and chemo-resistance by directly regulating tumor cell metabolism as an enzyme or regulating different signaling pathways as a nuclear transcription cofactor **(10)**.

PKM2 enhances tumor cell chemo-resistance. Increase of PKM2 production by alternative splicing promotes gemcitabine resistance in pancreatic cancer cells **(11)**. PKM2 modulates the sensitivity of colorectal cancer cells to gefitinib. Silencing PKM2 and kidney type glutaminase expression significantly reverses the resistance of colorectal cancer cells to oxaliplatin. Inhibition of PKM2 in esophageal squamous cell carcinoma cells significantly decreases cisplatin resistance and increases apoptosis by inactivating the pentose phosphate pathway **(12)**.

Independent of its pyruvate kinase activity, PKM2 exerts non-metabolic functions such as protein kinase activity and transcriptional co-activation. PKM2 has been observed to transfer to the nucleus, phosphorylate protein histone, and activate gene transcription, acting

non-metabolic functions in cancer cells **(13)**. The non-metabolic functions of PKM2 contribute to multiple processes of tumor pathology. In addition to tumor development, PKM2 is involved in cancer metastasis, epithelial mesenchymal transition (EMT), gene expression, mitosis, cellular proliferation, apoptosis, DNA damage response, and exosome secretion **(14)**.

High PKM2 expression is significantly associated with reduced overall survival in hepatocellular carcinoma. PKM2 promotes metastasis of hepatocellular carcinoma by recruiting myeloid-derived suppressor cells. PKM2 nuclear translocation and activation is required for the aerobic glycolysis-driven hepatocarcinogenesis **(10)**. In addition, knockdown of PKM2 in lung cancer suppresses tumor growth and invasion and enhances the efficacy of docetaxel and radiosensitivity. Pharmacologic activation of PKM2 by small molecules reduces lung tumor xenograft growth **(12)**.

PKM2 has appeared to be as a potential marker for pancreatic cancer diagnosis. The main advantages include that it detects a metabolic state specific for cancer cells, it can be measured easily in the blood, the outcomes are highly reproducible, with sufficient specificity and sensitivity, and its plasma concentrations are unaffected by cholestasis **(15).**

PKM2 levels were found to significantly higher in patients with pancreatic carcinoma, compared to those with chronic pancreatitis, non-neoplastic disease, or benign cystic pancreatic tumors. Although the diagnostic performance of PKM2 was lower than that of CA 19-9, the combination of PKM2 and CA 19-9 increased sensitivity of the diagnostic **(16).**

Unlike CA 19-9m PKM2 blood levels are unaffected by cholestasis. This may be due to the fact that CA 19-9 is also released by the biliary epithelium, while PKM2 is only expressed in actively proliferating cells. Patients with pre-operative tumour PKM2 values greater than 27 U/mL have a worse prognosis. Additionally, PKM2 serum levels were also related to tumour spread in pancreatic cancer patients **(15).**

Modulation of PKM alternative splicing in pancreatic ductal adenocarcinoma cells caused by frequent exposure to gemcitabine, conferring resistance to the drug. It is highly possible to combine gemcitabine and a PKM2 inhibitor in order to improve chemotherapeutic response in cancer pancreas **(16).**

**PKM2 as a therapeutic target:**

Up-regulation of PKM2 is a hallmark of numerous tumor types, making it a potential therapeutic target. Targeting PKM2 with some small molecules has been used in the preclinical studies to interfere with tumor growth. The strategy usually involves down-regulation of PKM2, blocking the nuclear translocation of PKM2 and promoting the tetrameric state of PKM2 **(17)**. For example, silencing PKM2 by specific siRNA increases tumor cell apoptosis and induces tumor regression in xenograft model **(14)**. LY294002, a specific phosphatidylinositol-3-kinase inhibitor, inhibits gastric cancer cell proliferation and induces early apoptosis through the down-regulation of PKM2. Beta-elemene, a drug for complementary cancer therapy, inhibits breast cancer metastasis via blocking PKM2 dimerization and nuclear translocation **(18)**.

Also, targeting PKM2 affects the sensitivity of cancer cells to chemotherapeutics. Knockdown of PKM2 increases 5-FU efficacy in colorectal cancer cells and the sensitivity of lung cancer xenograft to docetaxel. PKM2 knockdown or drug inhibition makes resistant hepatocellular carcinoma re-sensitize to doxorubicin and cisplatin **(Martin et al., 2020)**. Small molecule PKM2 activators may also interfere with the metabolism of cancer cells for therapeutic purposes **(13)**.

**Challenges against PKM2 protein kinase activity and its pro‑tumorigenic effects:**

Although accumulating evidence demonstrates protein kinase activity of PKM2, the conclusion has been challenged **(19)**. A phosphoproteomic survey has identified 974 PKM2 substrates in the proteome of renal cancer, but the biochemical evidence of PKM2 protein kinase activity is still limited. When recombinant PKM2 and 32P labelled PEP were added to PKM2-deficient cell lysates, neither PKM2-dependent phosphorylation nor PKM2-dependent transfer of phosphate from ATP directly to protein were observed. The results contradict a role of PKM2 as a protein kinase, although it may result from low levels of target substrate proteins and 32P-PEP in the reaction system **(20)**.

The role of PKM2 in tumorigenesis has been argued. A transgenic study revealed that PKM2 is not essential for BRCA1-deficiency-mediated breast cancer formation. In contrast, PKM2 deficiency without disrupting PKM1 accelerated breast cancer formation in a mouse model of BRCA1 deficiency. PKM2 is not necessary for cancer maintenance and growth in vivo **(21)**. Mice lacking PKM2 are prone to spontaneous development of hepatocellular carcinoma due to inflammation and an imbalance in metabolism **(22)**.

PKM1 has been revealed to be expressed in non-proliferating tumor cells but not in proliferating cells in PKM2 deficient tumors, suggesting that PKM2 is not required for cell proliferation and pyruvate kinase activity is necessary for non-proliferating tumor cells. These data demonstrated that cells can modify PKM2 activity to meet the metabolic requirements of proliferating and non-proliferating cancer cells. Discrepancies in results of PKM2 research may possibly be attributed to different experimental design or to cellular metabolic status **(23)**.

**Pyruvate Kinase M2 as prognostic and diagnostic marker in cancer:**

Metabolism reprogramming is the most important feature of the cancer cell. Tumor cells are different from normal cells as they depend on aerobic glycolysis to generate energy even if there is adequate oxygen in the surrounding (**13**). The cancer cell produces additional energy by increasing the reaction rate of glycolysis with the production of lactate in cytosol. In healthy cells, pyruvate is either wholly oxidized to $CO_2$ with more production of ATP in the presence of oxygen through the mitochondrial respiratory chain or converted to form lactic acid in oxygen-deficient conditions (**21**).

PKM2 promotes cancer development, metastasis and chemo-resistance by regulating either cancer cell metabolism directly as an enzyme or different signaling pathways as a nuclear transcription factor **(Figure 1)**, depending on the cancer type (**22**). PK catalyzes the final reaction of glycolysis, in which the high-energy phosphate group is transferred from phosphoenolpyruvate (PEP) to ADP to form Pyruvate, with the production of ATP **(23**).

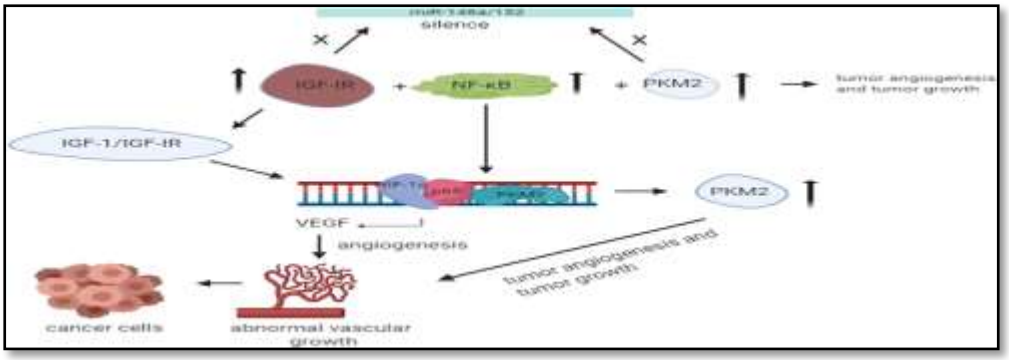


**Figure 1:** Interaction with PKM2, NF-kB subunit p65 activates the transcription of HIF-1α gene that increased secretion of VEGF translates, contributing to tumor growth [(22)].

PKM2 promotes the growth of gastric cancer cells by transcriptionally regulating Bcl-xL. PKM2 knockdown partially affected the stability of the NF-kB subunit p65, indicating that post-translational regulation of p65 is one of the mechanisms used by PKM2 to drive tumor growth **(22)**. Studies have reported presence of PKM2 in the blood of patients with gastrointestinal, pancreatic, lung and ovarian cancer, and renal cell carcinoma **(20-22)**.

Therefore, circulating PKM2 levels can be a potential diagnostic marker for these cancer types. The dimeric form of PKM2 in blood facilitated tumor growth and neo-angiogenesis by increasing endothelial cell proliferation, migration and adhesion to the extracellular matrix (**24**). The pro-proliferative functions of PKM2 depend on its nuclear translocation, which is promoted by different post-translational modifications like tyrosine phosphorylation, lysine acetylation, or sumoylation in response to epidermal growth factor receptor (EGFR), interleukin-3 (IL-3) and Oct-4, respectively. In addition to stimulating cell proliferation, PKM2 translocation also promotes apoptosis via a caspase and Bcl-2 independent manner in response to stimuli like DNA damage or oxidative stress **(25)**. Furthermore, PKM2 knockout in Eca109 and EC9706 cells activated caspase 3, down-regulated caspase 9, and increased expression of Bim **(26).**

**PKM2 facilitates tumor metastasis**

PKM2 is known to increase colorectal cancer metastasis by modulating the STAT3 signaling pathway. PKM2 mediated epithelial-mesenchymal transition (EMT), which is critical for cancer cells to acquire the invasive potential. Also, EMT stimulated the nuclear translocation of PKM2 and transcriptionally downregulated epithelial cadherin (a requirement for EMT induction). PKM2 also repressed E-cadherin epigenetically by interacting with the transcriptional factor TGF-β-induced factor homeobox 2, which induces histone H3 deacetylation and downregulates E-cadherin transcription. These findings indicate a novel regulatory axis involving PKM2 that can be utilized to prevent cancer metastasis **(27).**

- **PKM2 enhances chemo-resistance:**

Studies have linked dysregulated glucose metabolism with chemo-resistance, and since PKM2 is an important regulator of tumor glycolysis, it has been implicated in drug resistance in different GI cancers. 5-Fluorouracil (5-FU) is an important agent used for the systemic treatment of GI cancers, but has limited clinical response due to emergence of resistance among the patients. Studies have linked PKM2 expression and activity to cisplatin resistance in gastric tumor cells and to acquired resistance to 5-FU in CRC cells, indicating its role in

acquired chemo-resistance. In addition, PKM2 is a direct target of miR-122 in the colon cancer cells, and overexpression of the latter in 5-FU-resistant cells re-sensitizes them to 5-FU by inhibiting PKM2 **(19-22).**

The nucleoside analog gemcitabine (2',2'-difluorodeoxycytidine) is routinely used in the treatment of advanced pancreatic cancers. Nuclear translocation of PKM2 increases gefitinib resistance in colorectal cancer cells via STAT3 activation, and colorectal cancer patients who are resistant to gefitinib or oxaliplatin have higher PKM2 expression. In addition, knockdown of both PKM2 and GLS1 significantly reversed oxaliplatin-resistance in CRC cells. PKM2 also modulates the response of esophageal squamous cell carcinoma (ESCC) cells to chemotherapy by regulating the pentose phosphate pathway **(28).**

- **PKM2 & Biliary Tract Cancer**

PKM2 in bile and plasma can be used as a diagnostic and prognostic marker for biliary tract cancer, allowing early diagnosis of biliary tract cancer and for using PKM2 as a novel therapeutic target. PKM2 levels were significantly higher in biliary tract cancer patients. PKM2 levels were 9-fold higher in the bile of biliary tract cancer patients with comparison to patients with Benign Biliary Conditions **(1,29).**

A previous studies have reported that overexpression of PKM2 might act as a potential biomarker for specific types of cancer. However, it is not clearly understood how the expression of PKM2 changes the reaction of the cell upon the activation of the growth factor. The interaction of PKM2 with growth factor shows that the regulation is integrated. Intracellular ROS accumulation is also prevented by PKM2, thereby enabling the subsistence of cancer cells under oxidative stress **(30-32)**.

## Conclusion:

PKM2 may serve as an ideal therapeutic target since it is implicated in both glycolytic and non-glycolytic pathways and has a significant role in the carcinogenesis of tumour cells.

Bile PKM2 may be an acceptable biomarker for the detection of cancer in patients with unreliable biliary strictures.

PKM2 must therefore be further investigated in order to be a useful target for MBO therapy. Additionally, in order to successfully target PKM2 with medication, the level of PKM2 in cancer patients must be measured both qualitatively and quantitatively.

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## REFERENCES:

1- Gourgiotis, S., Kocher, H. M., Solaini, L., Yarollahi, A., Tsiambas, E., & Salemis, N. S. (2008). Gallbladder cancer. *The American Journal of Surgery*, *196*(2), 252-264.
2- Albores-Saavedra, J., Schwartz, A. M., Batich, K., & Henson, D. E. (2009). Cancers of the ampulla of vater: demographics, morphology, and survival based on 5,625 cases from the SEER program. *Journal of surgical oncology*, *100*(7), 598-605.
3- Hennedige, T. P., Neo, W. T., & Venkatesh, S. K. (2014). Imaging of malignancies of the biliary tract-an update. *Cancer imaging*, *14*(1), 1-21.

4- Alsowey, A. M., Salem, A. F., & Amin, M. I. (2021). Validity of MDCT cholangiography in differentiating benign and malignant biliary obstruction. *Egyptian Journal of Radiology and Nuclear Medicine*, *52*(1), 1-16.
5- Fernandez Y Viesca, M., & Arvanitakis, M. (2019). Early diagnosis and management of malignant distal biliary obstruction: a review on current recommendations and guidelines. *Clinical and Experimental Gastroenterology*, 415-432.
6- Ventrucci, M., Cipolla, A., Racchini, C., Casadei, R., Simoni, P., & Gullo, L. (2004). Tumor M2-pyruvate kinase, a new metabolic marker for pancreatic cancer. *Digestive diseases and sciences*, *49*, 1149-1155.
7- Xu, M. M., & Sethi, A. (2015). Diagnosing biliary malignancy. *Gastrointestinal Endoscopy Clinics*, *25*(4), 677-690.
8- Navaneethan, U., Lourdusamy, V., Poptic, E., Hammel, J. P., Sanaka, M. R., & Parsi, M. A. (2015). Comparative effectiveness of pyruvate kinase M2 in bile, serum carbohydrate antigen 19-9, and biliary brushings in diagnosing malignant biliary strictures. *Digestive Diseases and Sciences*, *60*, 903-909.
9- Dorrell, R., Pawa, S., Zhou, Y., Lalwani, N., & Pawa, R. (2020). The diagnostic dilemma of malignant biliary strictures. *Diagnostics*, *10*(5), 337.
10- Goonetilleke, K. S., Mason, J. M., Siriwardana, P., King, N. K., France, M. W., & Siriwardena, A. K. (2007). Diagnostic and prognostic value of plasma tumor M2 pyruvate kinase in periampullary cancer: evidence for a novel biological marker of adverse prognosis. *Pancreas*, *34*(3), 318-324.
11- Huguet, J. M., Lobo, M., Labrador, J. M., Boix, C., Albert, C., Ferrer-Barceló, L., ... & Sempere, J. (2019). Diagnostic-therapeutic management of bile duct cancer. *World journal of clinical cases*, *7*(14), 1732.
12- Wang, W. Q., Liu, W., Zhang, N., Wang, P. H., Cao, J. Z., Chen, J. M., ... & He, X. D. (2018). The Diagnostic Value of Pyruvate Kinase Isoenzyme Type M2 for Biliary Tract Carcinoma. A Systematic Review and Meta-Analysis. *Journal of Gastrointestinal & Liver Diseases*, *27*(1).
13- Dhar, D. K., Olde Damink, S. W., Brindley, J. H., Godfrey, A., Chapman, M. H., Sandanayake, N. S., ... & Pereira, S. P. (2013). Pyruvate kinase M2 is a novel diagnostic marker and predicts tumor progression in human biliary tract cancer. *Cancer*, *119*(3), 575-585.
14- Mohandas, K. M., Swaroop, V. S., Gullar, S. U., Dave, U. R., Jagannath, P., & DeSouza, L. J. (1994). Diagnosis of malignant obstructive jaundice by bile cytology: results improved by dilating the bile duct strictures. *Gastrointestinal endoscopy*, *40*(2), 150-154.
15- Bandara, I. A., Baltatzis, M., Sanyal, S., & Siriwardena, A. K. (2018). Evaluation of tumor M2-pyruvate kinase (Tumor M2-PK) as a biomarker for pancreatic cancer. *World Journal of Surgical Oncology*, *16*(1), 1-6.
16- Singh, A., Gelrud, A., & Agarwal, B. (2015). Biliary strictures: diagnostic considerations and approach. *Gastroenterology report*, *3*(1), 22-31.
17- Aguilar-Olivos, N. E., Oria-Hernández, J. A., Valdovinos-Andraca, F., Elizondo-Rivera, J., & Tellez-Avila, F. I. (2016). 119 Effectiveness of Biliary Pyruvate Kinase M2 for Diagnosis of Malignant Biliary Strictures: A Prospective and Comparative Study. *Gastrointestinal Endoscopy*, *83*(5), AB125.
18- Wu, J., Hu, L., Chen, M., Cao, W., Chen, H., & He, T. (2016). Pyruvate kinase M2 overexpression and poor prognosis in solid tumors of digestive system: evidence from 16 cohort studies. *OncoTargets and therapy*, 4277-4288.

19- Budzynska, A., Nowakowska-Dulawa, E., Marek, T., & Hartleb, M. (2016). Serum and Bile Insulin-Like Growth Factor I, Interleukin-6 and Tumor M2-Pyruvate Kinase in Differentiation Malignant from Benign Biliary Strictures–Preliminary Report. *Med Chem*, *6*, 429-434.

20- He, X., Du, S., Lei, T., Li, X., Liu, Y., Wang, H., ... & Wang, Y. (2017). PKM2 in carcinogenesis and oncotherapy. *Oncotarget*, *8*(66), 110656.

21- Wong, N., Ojo, D., Yan, J., & Tang, D. (2015). PKM2 contributes to cancer metabolism. *Cancer letters*, *356*(2), 184-191.

22- Azoitei, N., Becher, A., Steinestel, K., Rouhi, A., Diepold, K., Bobrovich, S., & Seufferlein, T. (2017). PKM2 promotes tumor angiogenesis by regulating HIF-1alpha through NF-kappaB activation. *Cancer Research*, *77*(13_Supplement), 1809-1809.

23- Zhang, W., Zhang, X., Huang, S., Chen, J., Ding, P., Wang, Q., ... & Hu, W. (2021). FOXM1D potentiates PKM2-mediated tumor glycolysis and angiogenesis. *Molecular Oncology*, *15*(5), 1466-1485.

24- Suzuki, H., Komuta, M., Bolog, A., Yokobori, T., Wada, S., Araki, K., ... & Kuwano, H. (2015). Relationship between 18-F-fluoro-deoxy-D-glucose uptake and expression of glucose transporter 1 and pyruvate kinase M2 in intrahepatic cholangiocarcinoma. *Digestive and Liver Disease*, *47*(7), 590-596.

25- Kumar, Y., Gurusamy, K., Pamecha, V., & Davidson, B. R. (2007). Tumor M2-pyruvate kinase as tumor marker in exocrine pancreatic cancer a meta-analysis. *Pancreas*, *35*(2), 114-119.

26- Aloysius, M. M., Zaitoun, A. M., Bates, T. E., Albasri, A., Ilyas, M., Rowlands, B. J., & Lobo, D. N. (2009). Complete absence of M2-pyruvate kinase expression in benign pancreatic ductal epithelium and pancreaticobiliary and duodenal neoplasia. *BMC cancer*, *9*(1), 1-8.

27- Chen, M., Liu, H., Li, Z., Ming, A. L., & Chen, H. (2021). Mechanism of PKM2 affecting cancer immunity and metabolism in Tumor Microenvironment. *Journal of Cancer*, *12*(12), 3566.

28- Liu, W. R., Tian, M. X., Yang, L. X., Lin, Y. L., Jin, L., Ding, Z. B., ... & Shi, Y. H. (2015). PKM2 promotes metastasis by recruiting myeloid-derived suppressor cells and indicates poor prognosis for hepatocellular carcinoma. *Oncotarget*, *6*(2), 846.

29- Xia, Y., Wang, X., Liu, Y., Shapiro, E., Lepor, H., Tang, M. S., ... & Wu, X. R. (2022). PKM2 is essential for bladder cancer growth and maintenance. *Cancer research*, *82*(4), 571-585.

30- Qian, Z., Hu, W., Lv, Z., Liu, H., Chen, D., Wang, Y., ... & Zheng, S. (2020). PKM2 upregulation promotes malignancy and indicates poor prognosis for intrahepatic cholangiocarcinoma. *Clinics and research in hepatology and gastroenterology*, *44*(2), 162-173.

31- Lu, W., Zhang, Y., Zhou, L., Wang, X., Mu, J., Jiang, L., ... & Liu, Y. (2016). miR-122 inhibits cancer cell malignancy by targeting PKM2 in gallbladder carcinoma. *Tumor Biology*, *37*(12), 15615-15625.

32- Zhu, H., Luo, H., Zhu, X., Hu, X., Zheng, L., & Zhu, X. (2017). Pyruvate kinase M2 (PKM2) expression correlates with prognosis in solid cancers: a meta-analysis. *Oncotarget*, *8*(1), 1628.