Expression and Significance of PKM1 in Acute Myeloid Leukaemia

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ABSTRACT

Objective: To investigate the expression level of pyruvate kinase M1 (PKM1) in patients with acute myeloid leukaemia (AML) as well as its clinical significance.

Study Design: A case-control study.

Place and Duration of the Study: Department of Haematology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, China, from January 2013 to 2023.

Methodology: The expression levels of PKM1 and pyruvate kinase m2 (PKM2) in the bone marrow of 65 AML patients (excluding M3) and 31 healthy volunteers were determined using reverse transcription-quantitative polymerase chain reaction (RT-qPCR), a method that measures fluorescence in real-time. The associations between PKM1, PKM2 expressions, clinical parameters, and the survival and prognosis of AML patients were analysed.

Results: AML patients showed higher PKM1 expression compared to controls. The area under the curve (AUC) of the receiver operating characteristics (ROC) was 0.65 ($p = 0.017$). PKM1 expression was correlated with peripheral blood leukocyte count ($r = -0.276$, $p = 0.026$), CCAAT enhancer-binding protein alpha *CEBPA* mutation ($r = -0.306$, $p = 0.014$), and chemotherapy-induced response ($r = -0.292$, $p = 0.018$). Patients with high PKM1 expression had a lower remission rate ($p = 0.019$) and long-term survival rate ($p = 0.034$) than those with low PKM1 expression. Patients with AML showed a rise in PKM2 levels; however, the variation was not statistically significant ($p > 0.05$).

Conclusion: PKM1 expression is upregulated in AML and patients with high PKM1 expression have a lower survival rate.

Key Words: *PKM1, Acute myeloid leukaemia, Clinical prognosis.*

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INTRODUCTION

AML is a condition characterised by the clonal growth of myeloid blasts in the blood system. The clinical symptoms and treatment outcomes of AML vary greatly.[1] In recent years, the application of arsenic trioxide has significantly improved the therapeutic effect of acute promyelocytic leukaemia. However, due to the lack of effective medicines for other types of AML, the prognosis of these types for patients is still very poor.[2] The emergence of AML, along with the development of medication resistance, and relapse is thought to be greatly influenced by metabolic reprogramming ultimately impacting the prognosis of AML through multiple factors.[3]

Cancer cells have very distinct metabolic features, shown by aerobic glycolytic metabolism, known as the Warburg effect, that differ from normal stem cells.[4,5]

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The ability of nearly all tumour cells to use glycolysis to break down glucose into energy, even when oxygen is present is an essential indicator of tumour metabolism. Although glycolysis is inferior to oxidative phosphorylation in terms of net ATP production, the cancer cells adapt to this less efficient metabolic mode.[6] Among the key enzymes of aerobic glycolysis, PKM is a key rate-limiting enzyme in glycolysis. Therefore, the expression of PKM has an important impact on the glucose metabolism and biochemistry of cells. In various haematological tumours, aerobic glycolysis has become a priority for tumour cells to obtain energy.[7] Currently, targeted therapies for glycolysis are undergoing clinical trials. There are two forms of PKM, PKM1 and PKM2 that may be used as biomarkers and predict tumour prognoses. Huang *et al*. found that increased levels of PKM2 in AML plasma are associated with an unfavourable prognosis.[8] Some studies indicate that PKM1 also plays a crucial part in aerobic glycolysis during tumorigenesis. For instance, PKM1 is significantly expressed in small cell lung cancer and the mechanism underlying this may involve the activation of autophagy.[9]

However, there is still uncertainty about the clinical relevance of PKM1 and PKM2 in non-M3 AML bone marrow. The objective of this study was to determine if PKM1 and PKM2 expression levels in patients with AML are clinically significant.

METHODOLOGY

From 2013 to 2016, a total of 65 bone marrow (BM) samples of AML patients diagnosed in the Department of Haematology, the First Affiliated Hospital of Guangxi Medical University, China, and 30 bone marrow samples of healthy people were collected. AML inclusion criteria were patients with types of AML other than AML-M3: Who were diagnosed for the first time and did not receive chemotherapy. Patients with other malignant tumours, AML transformed from myeloproliferative neoplasms (MPN) or myeloproliferative disorder (MPD), or treatment-related AML were excluded. Inclusion criteria for healthy people were those without abnormal blood routine and bone marrow morphology and no major infectious diseases. In all the experimental and normal groups, the anterior superior iliac spine or posterior superior iliac spine was used as the puncture point and routine bone marrow puncture was performed, and about 4ml of bone marrow fluid was extracted. The AML patients included in the study were regularly followed up by telephone to observe their physical condition and treatment effect until January 2023. All participants provided written informed consent according to the Declaration of Helsinki.

Every individual contributed 2ml of BM specimens which were subsequently subjected to density gradient centrifugation for the separation of BM monocytes. BM cells were employed for the isolation of entire RNA, adhering to the guidelines of the manufacturer and using a trizol reagent (Invitrogen, USA). Afterwards, the RNA underwent conversion into cDNA by the means of reverse transcription. Superscript™ III reverse transcriptase (Invitrogen) was used for the reverse transcription process on the applied biosystem geneamp™ PCR system 9700. The applied biosystems ViiA 7 real-time PCR system and the arraystar 2X PCR master mix kit were used for conducting qRT-PCR. The qRT-PCR procedure commenced with an initial denaturation step at a temperature of 95°C for a period of 10 minutes. Afterwards, there were 40 rounds of PCR, where each round involved 10 seconds at 95°C and annealing at a temperature of 60°C for one minute. The primers employed in this study include the *β-actin* forward primer (sequence: 5'-gtggcgaggactttgattg-3') and reverse primer (sequence: 5'-cctgtaacacgcatctcatatt-3'), the PKM1 forward primer (sequence: 5'-cgagcctcaagtcactcactccac-3'), and reverse primer (sequence: 5'-gtgaggagcaccttgccagact-3'), and the PKM2 forward primer (sequence: 5'-attatttgaggaactccgccgcct-3'), and reverse primer (sequence: 5'-attccggtcagcaatgatgg-3'). *β-actin* genes were used as housekeeping genes. The CT method and the 2-ΔΔ calculation were used to estimate the relative gene levels of PKM1 and PKM2.

The Kolmogorov-Smirnov test and the Shapiro-Wilk test were used to test the normality of the data. Normal data were compared by the parametric t-test and non-normal data were compared by the Mann-Whitney U-test. The Mann-Whitney U-test was employed to examine the comparative manifestation of PKM1 and PKM2 within various groups, taking into account the deviation of the data from the standard distribution. Median (IQR) was used to represent quantitative variables. Based on PKM1 expression, AML patients were categorised into low expression and high expression groups. The middle average value of PKM1 was utilised as the standard for the division. Qualitative variables were presented as frequency and percentage. The comparison between PKM1 expression and clinical data was conducted using either Fisher's exact probability or Pearson's Chi-square test. In addition to gender, age, haemoglobin, etc., the clinical data of patients were also analysed for fab-typing, karyotype, and several mutations (NPM1, flt3-itd, c-kit, dnmt3, and *CEBPA*). For the variables with differences between the two groups, the Pearson correlation coefficient or Spearman rank correlation coefficient method was further used to calculate the correlation between the two variables. The r value was used to assess the linear relationship between two variables. The assessment of the diagnostic significance of PKM1 expression in bone marrow was conducted by analysing ROC curves, AUCs, sensitivity, and specificity. To examine the correlation between PKM1 expression and clinical outcomes, a Kaplan-Meier survival analysis was conducted. The study utilised the Cox proportional hazard regression model to analyse the statistically significant factors associated with risk in AML patients' clinical prognoses. The statistical analysis was performed utilising SPSS 26.0 software (SPSS Inc, Chicago, IL, USA) and Graphpad Prism 9 software (Grapghpad Software Inc, La Jolla, CA, USA). Results were considered statistically significant when the p-value was below 0.05.

RESULTS

Table I provides a summary of the clinical characteristics of the patients. A control group consisting 31 healthy volunteers was used. The bone marrow of AML patients showed significantly higher levels of PKM1 expression compared to the controls as indicated by the real-time fluorescence PCR data ($p = 0.017$, Figure 1A). According to the ROC curve analysis, the value of AUC was 0.652 (AUC = 0.652, 95% CI = 0.541-0.762, in Figure 2). Although the AUC value of 0.652 was lower than the commonly considered threshold of "good" (for example, 0.7 or higher), it still showed that this indicator had a certain discriminatory ability compared to random guess (AUC = 0.5). Nevertheless, there was no notable disparity in the PKM2 expression levels between the patients and controls ($p > 0.05$, Figure 1B). This may mean that PKM1 plays a more important role in the disease or that its expression level is more directly related to the development and progression of the disease, so the clinical correlation analysis, univariate analysis, and multivariate analysis of PKM1 were carried out. Based on the median level of PKM1 expression the patients were divided into two groups: Low PKM1 expression (n = 33) and high PKM1 expression (n = 32). Table I shows the correlation between PKM1 expression and clinical features. There was an inverse relationship between expression levels of PKM1 and peripheral blood leukocyte count ($r = -0.276$, $p = 0.026$), *CEBPA* mutations ($r = -0.306$, $p = 0.014$), and induced chemotherapy responses ($r = -0.292$, $p = 0.018$).

Table I: The relationship between clinical and molecular characteristics of AML patients and PKM1 expression.

Clinical characteristics	PKM1 mRNA level		p-value
	Low (n = 33)	High (n = 32)	
Gender, n (%)			0.702
Male	16 (24.6)	14 (21.5)	
Female	17 (26.2)	18 (27.7)	
Age, years, median (IQR)	31 (22-69)	39 (20 - 60)	0.178
Hb(g/L), median, (IQR)	75.6 (46.0 - 124.0)	73 (43.0 - 124.0)	0.843
WBC, x10^9/L median (IQR)	19.69 (1.72 - 286.00)	10.18 (0.09 - 246.00)	0.035
PLT, x10^9/L median (IQR)	39.00 (9.40 - 236.20)	36.65 (7.00 - 168.00)	0.694
BM blasts, %, median (IQR)	55.0 (2.0 - 94.5)	55.5 (10.0 - 96.0)	0.968
FAB type, n (%)			0.481
M1	4 (6.2)	1 (1.5)	
M2	7 (10.8)	9 (13.8)	
M4	12 (18.5)	14 (21.5)	
M5	8 (12.3)	8 (12.3)	
M6	2 (3.1)	0 (0)	
Chromosome karyotype, n (%)			0.057
Normal	21 (32.3)	27 (41.5)	
Abnormal	12 (18.5)	5 (7.7)	
Mutation status, n (%)			
NPM1 mutation			0.349
	7 (10.8)	4 (6.2)	
Wild type	26 (40.0)	28 (43.1)	
FLT3-ITD mutation			0.783
	6 (9.2)	5 (7.7)	
Wild type	27 (41.5)	27 (41.5)	
C-kit mutation			0.584
	1 (1.5)	3 (4.6)	
Wild type	32 (49.2)	29 (44.6)	
DNMT3 mutation			0.371
	4 (6.2)	1 (1.5)	
Wild type	29 (44.6)	31 (47.7)	
CEBPA mutation			0.035
	8 (12.3)	1 (1.5)	
Wild type	25 (38.5)	31 (47.7)	
Complete remission, n (%)			0.019
Yes	22 (33.8)	12 (18.5)	
No	11 (16.9)	20 (30.8)	

The Mann-Whitney U-test, Fisher's exact probability, and Pearson's Chi-Square test were used to compare the high and low PKM1 groups.

Table II: Univariate and multivariate analyses in AML patients.

Variables	Univariate		Multivariate	
	hazard ratio (95% CI)	p-value	hazard ratio (95% CI)	p-value
PKM1 (low *vs.* high)	1.875 (1.031-3.410)	0.039	1.914 (1.052-3.481)	0.034
Gender (female *vs.* male)	1.707 (0.947-3.076)	0.075		
Age (years)	1.007 (0.984-1.032)	0.544		
Leukocyte, x10^9/L (≤20 *vs.* >20)	1.011 (0.557-1.837)	0.971		
BM blasts (%) (≤60 *vs.* >60)	1.847 (1.022-3.338)	0.042	1.886 (1.043-3.412)	0.036
NPM1 (wild *vs.* mutated)	0.540 (0.228-1.278)	0.161		
FLT3–ITD (wild *vs.* mutated)	1.194 (0.555-2.567)	0.650		
C-kit (wild *vs.* mutated)	1.281 (0.395-4.157)	0.680		
DNMT3 (wild *vs.* mutated)	0.419 (0.101-1.732)	0.230		
CEBPA (wild *vs.* mutated)	1.303 (0.581-2.921)	0.520		

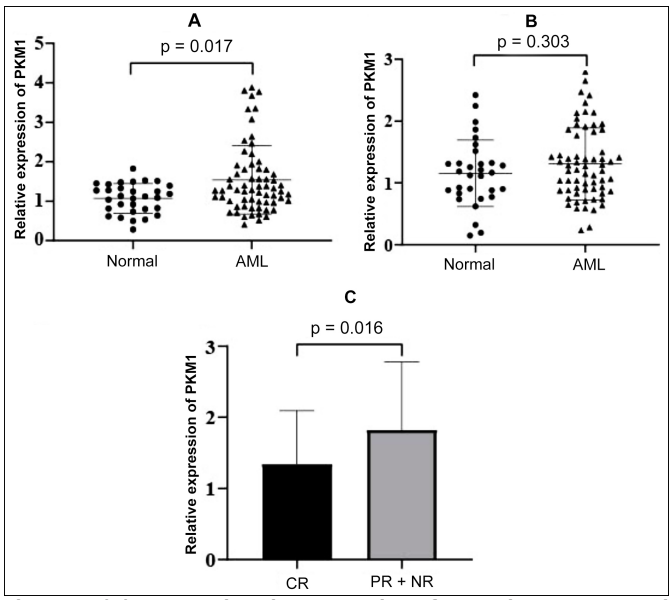

Figure 1: (A) Contrasting the expression of PKM1 between normal controls and the AML group. (B) Evaluating the relative expression of PKM2 in normal controls and the AML group. (C) Comparison of PKM1 expression in different-induced chemotherapy responses.

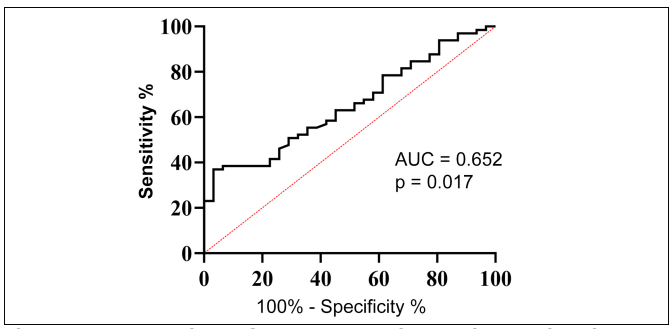

Figure 2: A comparison of AML cases and normal controls using ROC analysis using PKM1.

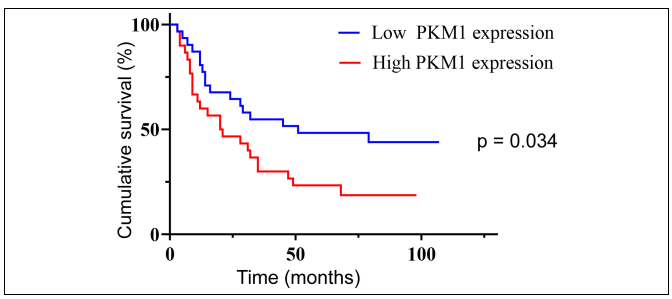

Figure 3: Two groups were formed among AML patients, categorised according to the median expression level of PKM1, and survival curves were created using the Kaplan-Meier method.

Based on the patients' initial response to remission induction therapy, the complete response (CR), and partial response (PR)+ no response (NR) groups were identified. The CR group had considerably lower PKM1 expression compared to the PR + NR group (p = 0.016, Figure 1C). The connection between PKM1 expression and overall survival (OS) in AML patients was confirmed using the product limit approach to assess the association between PKM1 and the outcome. There was a notable disparity in OS between the cohorts displaying low and high PKM1 expression (p = 0.034; Figure 3). Patients diagnosed with AML and exhibiting reduced PKM1 expression demonstrated a higher rate of OS survival. Furthermore, multivariate analysis indicated that the level of PKM1 expression could serve as a reliable predictor for OS (p = 0.034, Table II).

DISCUSSION

AML is a highly heterogeneous malignant haematological disease. The development of this condition involves the interaction of several genes. Although the precise mechanism is yet unknown, it is believed that it is brought on by the uncontrolled proliferation, differentiation, and death of bone marrow primordial cells with chromosome abnormalities or gene mutations. Changes in the tumour micro-environment may cause cancer cells to become resistant to therapeutic drugs. However, metabolic reprogramming of tumour cells affects the tumour micro-environment all the time. Some studies have shown that AML resistance is related to increased glycolytic activity.[10]

The main focus of this research is on the pyruvate kinase PKM, which plays a role in the metabolism of glucose. PKM produces two forms PKM1 and PKM2. PKM1 is involved in oxidative phosphorylation, whereas PKM2 is involved in glycolysis. Due to the heterogeneous characteristics of tumours, the roles of PKM1 and PKM2 can differ in various cancer types occasionally exhibiting contradictory functions. In order to explore potential targets for cancer treatment, it would be advantageous to examine the quantities of PKM1 and PKM2 present in cancerous cells and observe how their expression fluctuates throughout the course of time. Researches have shown that PKM2 levels are increased in pancreatic ductal adenocarcinoma, gastric cancer, liver cancer, colorectal cancer, oesophageal squamous cell carcinoma, gallbladder cancer, and various other tumours resulting in an adverse prognosis.[11-13] Some studies suggest that PKM1 transforms into PKM2 during tumour formation, thus promoting the development and progression of tumours.[14] However, Desai *et al*. found that only glioblastoma showed PKM1 to PKM2 transformation among 16 tumour samples analysed, including pancreatic cancer, breast cancer, glioblastoma, and other tumours.[15] Similarly, Bluemlein *et al*. conducted a study using 25 tumour samples and certain cell lines but found no evidence of PKM1 transforming into PKM2.[16] These results seem to differ from previous studies, so which form of PKM do tumour cells choose to express in order to proliferate and grow? Israelsen *et al*. discovered that mice lacking PKM2 had increased tumour growth after PKM2 knockout possibly due to compensatory PKM1 expression.[17] Chiavarina *et al*. found that PKM1 in the extracellular matrix feeds tumour cells and triggers tumour inflammation by increasing L-lactate secretion.[18] Prakasam *et al*. observed that knocking down PKM1 in the lung cancer cell line A549 enhanced apoptosis.[19] Okazaki *et al*. found that PKM1 expression levels were upregulated in drug-resistant gastric cancer cell lines.[20] In drug-resistant tumour cells, Taniguchi *et al*. observed a significant increase in PKM1 levels while PKM2 expression

remained low. Furthermore, a robust correlation was observed between PKM1 and oxidative phosphorylation (OXPHOS); the suppression of PKM1 led to an enhanced susceptibility of cancer cells to medications.[21] These studies showed that PKM1 is involved in tumour progression and cancer cell resistance to chemotherapeutic drugs.

In this study, compared with the normal control group, the bone marrow in AML patients' PKM1 expression increased obviously and no obvious difference was found between PKM2 expression. A larger first remission rate was associated with relatively low PKM1 expression than it was with relatively high expression and vice versa. In addition, patients in high PKM1 expression groups had a poorer survival rate. The present results suggested that increased PKM1 expression in bone marrow may be a factor in AML tumour treatment resistance.

Reactive oxygen species were discovered to be in relatively low concentrations in AML stem cells, according to Lagadinou *et al*. After inhibiting Bcl-2, OXPHOS was reduced, leading to the elimination of static leukaemia stem cells (LSCs), thereby reducing the recurrence of AML.[22] Currently, the role of PKM1 in AML remains unclear. From the point of view of this study, the high expression of PKM1 may lead to the upregulation of OXPHOS which is beneficial to AML stem cells. Furthermore, it is conceivable that PKM1 influences AML's metabolism of glucose promoting tumour development. It is important to keep researching how PKM1 and OXPHOS interact in AML bone marrow in order to find novel treatment targets for the disease. The limitation of this study was that the sample size was small and it was a single-centric study which may result in certain regional limitations on the applicability of the findings.

CONCLUSION

In comparison to the healthy control group, the expression of PKM1 in the bone marrow of AML patients increased dramatically, and patients with relatively high PKM1 expression had a shorter survival rate than patients with relatively low PKM1.

ETHICAL APPROVAL:

The Human Ethics Committee at The First Affiliated Hospital of Guangxi Medical University, Guangxi, China, approved this study (Approval no: 2022-E417-01).

PATIENTS' CONSENT:

All patients provided written informed consent.

COMPETING INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

SZ, ZL: Idea or design for the work.
SZ, XH, YY, ZT: Acquisition and analysis of the data.
SZ, XL, XH, YY, ZT: Work on the draft.
XL, ZL: Approval of the final version.
All authors approved the final version of the manuscript to be published.

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