RESEARCH ARTICLE

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Prognostic Value of *PKM2* Expression in de novo Metastatic Colorectal Cancer Patients

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Abstract

Background: Metastatic colorectal cancer (CRC) is considered one of the lethal neoplasms globally. The embryonic M2 isoform of pyruvate kinase (*PKM2*), a crucial enzyme regulating glycolysis, promotes tumor cell proliferation and metastasis, and is correlated with unfavourable outcomes among several cancers, including CRC. **Methods:** This prospective cohort study included 80 de novo metastatic CRC patients at the Medical Oncology Department, South Egypt Cancer Institute (SECI), Assiut University, from June 2022 to June 2024. We analyzed the impact of *PKM2* expression, evaluated by immunohistochemistry (IHC), in metastatic CRC patients, and response to first-line oxaliplatin-based chemotherapy regimens and survival outcomes in the form of progression-free survival (PFS) and overall survival (OS). **Result:** The median age of the studied cases was 47 ys, 42 (52.5%) were male, and 54 (67.5%) were left-sided. Our results revealed a statistically significant association between high *PKM2 IHC* expression and lower overall response rate (ORR), P = 0.001. After a median follow-up time of 13 months(ms), this translated into a statistically significant association between the high *PKM2 IHC* expression group and lower PFS and OS (6ms vs 10ms; P = 0.008), (12ms vs. 17ms; P = 0.008), respectively. **Conclusion:** Our results concluded that *PKM2 IHC* expression was one of the independent prognostic factors for OS. This supports the consideration of *PKM2* as a promising molecular target for therapeutic strategies in CRC management.

Keywords: oxaliplatin- response- progression-free survival- overall survival

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Introduction

CRC is the third most frequently diagnosed cancer worldwide and the second leading cause of cancer-related mortality. In 2022, around 1.9 million people were diagnosed with CRC, and more than 900,000 deaths were related to CRC [1]. In Egypt, CRC ranks as the 7th most common cancer, representing 3.47% of cancers in men and 3% in women [2].

Although 70–80% of early colon cancer cases show promise for curative surgical resection, almost half of them will experience metastasis [3]. Approximately 20% of CRC patients presented with distant metastases [4]. Metastasis is the main contributor to mortality from CRC [5]. Although the introduction of new cytotoxic and biologic therapies, anti-epidermal growth factor (EGFR), and anti-vascular endothelial growth factor (VEGF), has significantly improved patient prognosis [6]. oxaliplatin, a chemotherapeutic platinum-based agent, remains a cornerstone in the treatment of metastatic CRC [7].

PKM2 is an isoform of the glycolytic enzyme, pyruvate kinase, which catalyzes the final step of glycolysis, converting the phosphoenolpyruvate to pyruvate, leading to net ATP production, a universal energy carrier in cells, during the glycolytic sequence [8]. Beyond its classical role in glycolysis, *PKM2* has been implicated in various cellular processes, including gene transcription and regulation of the cell cycle [9, 10]. *PKM2* has also been reported to be associated with tumorigenesis [11]. Furthermore, *PKM2* contributes to tumor angiogenesis by activating nuclear factor kappa B (NF-κB) and hypoxia-inducible factor 1-alpha (HIF-1α) [12].

PKM2 is widely expressed in various cancer types [13]. Nevertheless, its role in CRC is controversial. While some studies have reported that *PKM2* expression was increased in colon cancer cells, they also found that its upregulation was associated with advanced stages [14]. In contrast, some studies have demonstrated that *PKM2* may have a minimal role in colon cancer initiation or progression or that its loss may even promote colon cancer [15, 16]. Conflicting evidence exists regarding the relationship between high *PKM2* expression and the efficacy of platinum-based regimens across various cancers. Some studies, such as those on cervical cancer, suggest that increased *PKM2* expression may enhance platinum chemosensitivity [17]. However, other studies

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have demonstrated that its upregulation has been linked to platinum resistance in different cancers, like osteosarcoma, non-small cell lung cancer, and CRC [17–19]. This discrepancy prompted us to explore its prognostic value and therapeutic potential in metastatic CRC.

The Aim of this study was to evaluate the impact of *PKM2 IHC* expression on response and survival in metastatic CRC patients receiving first-line treatment with oxaliplatin-based regimens.

Materials and Methods

Study design and patients

A prospective cohort study of 80 de novo stage IV CRC patients, as defined by the Eighth Edition of the American Joint Committee on Cancer (AJCC) staging system for colon cancer [20], received first-line treatment with oxaliplatin-based regimens (FOLFOX, CAPOX) at the Medical Oncology Department, SECI, Assiut University, from June 2022 to June 2024. The inclusion criteria were patients aged 18 years (ys) or older, of any gender, with histologically confirmed colorectal adenocarcinoma. Eligible Participants were required to have documented synchronous stage IV CRC, an Eastern Cooperative Oncology Group (ECOG) performance status of 2 or lower, and adequate hematological, liver, and renal function. Patients with double malignancy, pregnant and lactating patients, seriously uncontrolled concomitant disease, and metachronous stage IV CRC were excluded. Ethical approval for the study was granted by our institutional ethical committee, the SECI-Institutional Review Board (IRB), under approval number IORG0006563-602.

Assessment of metastatic CRC status

Participants in this study were subjected to clinical examination, complete laboratory investigations, and pathological examination. Rat sarcoma (RAS) and V-Raf Murine Sarcoma Viral Oncogene Homolog B (BRAF) gene expressions were evaluated using polymerase chain reaction (PCR) on tissue biopsies, and *PKM2* protein expression was assessed via IHC. Chest, abdominal, and pelvic computed tomography (CT) scans were conducted at diagnosis, every three months during treatment, and every six months during the follow-up period. Treatment response was interpreted based on the response evaluation criteria in solid tumours version 1.1 (RECIST 1.1) guidelines [21].

OS was defined as the duration from the start of treatment to the time of death from any cause. PFS was defined as the time from treatment initiation until the first documented tumour progression or death. ORR was defined as the percentage of patients who achieved complete response (CR), partial response (PR), or stable disease (SD) as determined by the investigator.

PKM2 IHC

Formalin-fixed paraffin-embedded (FFPE) tissue blocks were sectioned into 4μ slices and mounted on positively charged slides. The tissue sections were then deparaffinized with xylene and rehydrated using a graded series of ethanol solutions in descending concentrations. Antigen retrieval was performed using a high PH Tris-EDTA solution at 97 °C for 20 minutes. Hydrogen peroxide block was applied for 5 min, then washed with PBS. Incubation with primary antibody (*PKM2* Rabbit polyclonal Ab, A13905, ABclonal, USA) at a concentration of 1/100 for one hour. Universal staining kit "PolyQ stain 2 step detection system goat Anti-mouse/rabbit HRP, peroxidase quench, DAP-kit (Ready-To-Use)" (Protaqs® Quartett, catalog # DK-211-015, BIOCYC Gesellschaft für Biotechnologie, Kosmetik und Recyclingverfahren mbH & Co. Entwicklung KG Am Mühlenberg 11, 14476 Postdam, Germany) was applied at room temperature for one hour following the manufacturer's instructions. After three washes with PBS, the staining was visualized using 3, 3'-diaminobenzidine tetrahydrochloride (DAB), followed by counterstaining with Mayer's haematoxylin.

Evaluation of PKM2 expression

Positive cytoplasmic staining of *PKM2* was considered positive staining. A known case of colon cancer with positive *PKM2* expression was used as a positive control. Scoring was calculated based on the positive area: less than 5% received a score of 0, 6-25% received a score of 1, 25-50% received a score of 2, and more than 50% received a score of 3. A staining intensity score of 0 was assigned to no staining, 1 to weak staining, 2 to moderate staining, and 3 to strong staining. Multiplying the intensity score by the percentage score yielded the final expression level. A total score below 4 was classified as low expression, while a score of 4 or higher indicated high expression [22].

Statistical analysis

All statistical calculations were conducted using SPSS software, version 26 (Statistical Package for the Social Sciences; SPSS Inc., Chicago, IL, USA). Quantitative data were presented as mean ± standard deviation (SD) or median (range). Qualitative data were expressed as frequencies and percentages as applicable. Comparison of quantitative variables was carried out using the Student t-test. For comparing categorical variables, the Chi-square (χ^2) test was applied, and when expected cell counts were below 5, Fisher's Exact test was utilized. PFS and OS were analyzed using the Kaplan-Meier method, with differences assessed via the log-rank test. To evaluate predictors of ORR in metastatic CRC patients, odds ratios (OR) with 95% confidence intervals (CI) and logistic regression analysis were employed. Cox proportional hazards regression was used to identify factors influencing PFS and OS among metastatic CRC cases. P-value is always 2 tailed and set significant at <0.05.

Results

Demographics and clinicopathological characteristics

The median age among studied patients was 47 (range 20-80 ys) for both genders. Forty-two (52.5%) were males, twenty-six (32.5%) were right-sided, while fifty-four (67.5%) were left-sided. We categorized metastatic patients into M1a, M1b, and M1c according to the AJCC, Eighth Edition [20]) which were found to be 32(40%),

13(16.25%), and 35(43.75%), respectively.

Regarding pathological criteria, thirty-two (40%) were with mucinous differentiation, eighteen cases (22.5%) were RAS mutated, while only three cases (3.75%) were BRAF mutated. Table 1 presents an overview of the main demographic and clinicopathological characteristics of the study population.

Correlation between PKM2 expression and clinicopathological characteristics

Regarding *PKM2* expression, it was found that 38(47.5%) and 42(52.5%) had *PKM2* low and high expression, respectively. Expression of *PKM2* in colorectal tumour cells is depicted in Figure 1. High *PKM2* expression was significantly associated with a lower median age (P = 0.001) and a higher frequency of RAS mutations (P = 0.013). Additionally, there was a borderline significant correlation between *PKM2* expression and mucinous differentiation (P = 0.05). However, no statistically significant associations were observed between *PKM2* expression and other factors such as gender, tumor location, BRAF status, or M stage, as presented in Table 2.

Response rate and PKM2 expression

We observed that low *PKM2* expression was associated with a higher percentage of ORR 65.1%, while high expression was associated with a lower percentage of ORR 34.9% (P = 0.001). Figure 2 shows how *PKM2* expression and ORR are correlated.

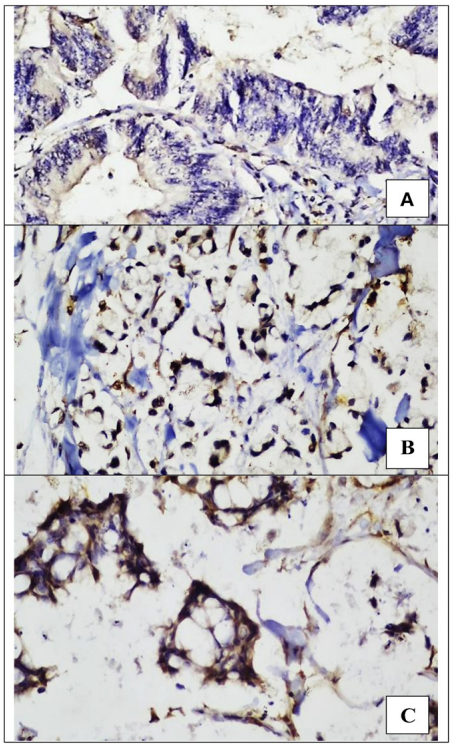

Figure 1. Expression of *PKM2* in Colorectal Tumor Cells (A) Low *PKM2* expression in well-differentiated adenocarcinoma (x 40). (B) High *PKM2* expression in signet ring cell carcinoma (x 20). (C) High *PKM2* expression in mucinous adenocarcinoma (x40).

Survival outcomes concerning PKM2 IHC expression

Our study demonstrated statistically significant lower PFS (6 ms, 95% CI: 5.22–6.77 ms) in high *PKM2* expression cases compared to those with low *PKM2* expression (10 ms, 95% CI: 7.99–12.003 ms; P = 0.008).

Similarly, shorter OS was significantly correlated with high *PKM2* expression (12 ms, 95% CI: 9.36-14.63 ms) versus (17 ms, 95% CI: 11.82-22.17) for low *PKM2 IHC* expression (P =0.008) after a median follow-up time of 13 ms for the whole study. Figure 3 illustrates the PFS and OS survival curves according to *PKM2* expression.

Predictors of response and survival in univariate and multivariate analysis

Response rate

In multivariate analysis, *PKM2 IHC* expression (OR:

Table 1. Demographic and Clinicopathological Criteria of the Studied Metastatic CRC Patients (n=80)

Variable	
Age	
Mean±SD	46.22±15.34
Median (Min- Max)	47 (20-80)
≤60	66 (82.5%)
>60	14 (17.5%)
Gender	
Male	42 (52.5%)
Female	38 (47.5%)
Location	
Right-sided	26 (32.5%)
Left-sided	54 (67.5%)
Regimen	
Capox	58 (72.5%)
Folfox	22 (27.5%)
BRAF status	
Wild	51 (63.75%)
Mutant	3 (3.75%)
Unknown	26 (32.5%)
RAS status	
Wild	52 (65%)
Mutant	18 (22.5%)
Unknown	10 (12.5%)
Median number of metastatic sites	1 (1-4)
M Stage	
M1a	32 (40%)
M1b	13 (16.25%)
M1c	35 (43.75%)
Histology	
Mucinous	32 (40%)
Non-mucinous	48 (60%)
PKM2 expression	
Low	38 (47.5%)
High	42 (52.5%)

Quantitative data are presented as mean ± SD and median (range), and qualitative data are presented as frequencies (percentages).

Table 2. Demographic and Clinicopathological Characteristics According to *PKM2* Expression (n=80):

Variable	*PKM2*		
	Low	High	P-value
Age median(min-max)	52 (20-73)	38.5 (22-80)	0.001*
Age			
≤60	29 (76.3%)	37 (88.1%)	0.166
>60	9 (23.7%)	5 (11.9%)	
Gender			
Male	18 (47.4%)	24 (57.1%)	0.382
Female	20 (52.6%)	18 (42.9%)	
Location			
Right-sided	15 (39.5%)	11 (26.2%)	0.205
Left-sided	23 (60.5%)	31 (73.8%)	
Histology			
Mucinous	11 (28.9%)	21 (50%)	0.055
Non-mucinous	27 (71.1%)	21 (50%)	
RAS status			
Wild	32 (86.5%)	20 (60.6%)	0.013*
Mutant	5 (13.5%)	13 (39.4%)	
BRAF status			
Wild	31 (91.2%)	20 (100%)	0.287
Mutant	3 (8.8%)	0	
M stage			
M1a	16 (42.1%)	16 (38.1%)	0.744
M1b	7 (18.4%)	6 (14.3%)	
M1c	15 (39.5%)	20 (47.6%)	

Quantitative data are presented as median (range), and qualitative data are presented as frequencies (percentages). Student t-test was used to compare quantitative variables. Chi-square test or Fisher's Exact test was used to compare categorical data. *Significance defined by $p < 0.05$.

6.402, 95% CI: 2.107-19.453; P = 0.001) stood as the most powerful independent variable and predictor of ORR.

PFS

In multivariate analysis, M stage and age have emerged as the strongest independent predictors of PFS. Patients with M1b disease had a hazard ratio (HR) of 5.53 (95% CI: 1.65–18.47; P = 0.005), while those with M1c stage showed an HR of 2.94 (95% CI: 1.11–7.81; P = 0.03), and patients aged 60 years or older had a reduced risk with a HR of 0.147 (95% CI: 0.029–0.746; P = 0.021). After adjusting for other variables, *PKM2 IHC* expression, mucinous differentiation, and BRAF mutation status were no longer statistically significant in predicting PFS.

OS

In multivariate analysis, *PKM2 IHC* expression (HR: 2.545, 95% CI: 1.325–4.888; P = 0.005) and mucinous

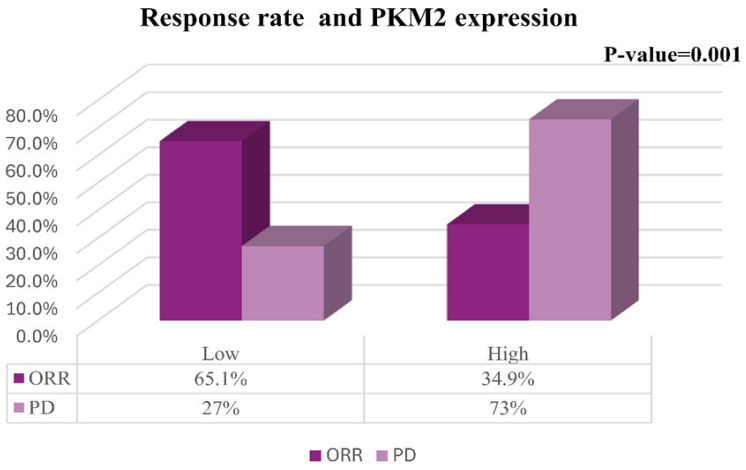

Figure 2. Response Rate and *PKM2 IHC* Expression

Table 3. Univariate and Multivariate Analysis of 80 Patients with Metastatic CRC

	ORR			PFS			OS		
	OR	95% CI	P-value	HR	95% CI	P-value	HR	95% CI	P-value
Univariate analysis									
Age (years)									
< 60	Ref			Ref			Ref		
≥ 60	0.4	(0.114-1.404)	0.153	0.282	(0.113-0.707)	0.007*	1.308	(0.673-2.542)	0.428
Location									
Right-sided	Ref			Ref			Ref		
Left-sided	2.03	(0.772-5.35)	0.151	1.176	(0.670-2.063)	0.573	1.074	(0.620-1.859)	0.799
Gender									
Male	Ref			Ref			Ref		
Female	0.727	(0.301-1.76)	0.48	0.829	(0.500-1.375)	0.468	0.643	(0.379-1.089)	0.1
Mucinous									
Yes	3.039	(1.201-7.691)	0.019*	2.33	(1.384-3.923)	0.001*	2.501	(1.485-4.214)	0.001*
No	Ref			Ref			Ref		
RAS status									
Wild	Ref			Ref			Ref		
Mutant	2.51	(0.837-7.55)	0.1	1.846	(0.993-3.432)	0.053	1.055	(0.565-1.971)	0.867
BRAF status									
Wild	Ref			Ref			Ref		
Mutant	3.1	(0.263-36.481)	0.368	3.533	(1.05-11.88)	0.042*	1.125	(0.269-4.704)	0.872
M stage									
M1a	Ref			Ref			Ref		
M1b	6.75	(1.62-28.03)	0.009*	2.288	(1.058-4.947)	0.036*	3.304	(1.659-6.986)	0.001*
M1c	4	(1.40-11.35)	0.009*	3.074	(1.686-5.604)	0.000*	1.512	(0.831-2.749)	0.175
PKM2 expression									
Low	Ref			Ref			Ref		
High	5.04	(1.93-13.15)	0.001*	1.878	(1.121-3.146)	0.017*	1.974	(1.154-3.378)	0.013*
Regimen									
Capox	Ref			Ref			Ref		
Folfox	0.957	(0.357-2.56)	0.93	0.909	(0.519-1.592)	0.739	0.964	(0.534-1.742)	0.904
Multivariate analysis									
Age (years)									
< 60				Ref					
≥ 60				0.147	(0.029-0.746)	0.021*			
BRAF status									
Wild				Ref					
Mutant				2.947	(0.767-11.324)	0.116			
Mucinous									
Yes	1.413	(0.415-4.809)	0.581	1.203	(0.473-3.061)	0.698	2.616	(1.424-4.807)	0.002*
No	Ref			Ref			Ref		
M Stage									
M1a	Ref			Ref			Ref		
M1b	10.27	(1.964-53.710)	0.006*	5.534	(1.658-18.477)	0.005*	5.735	(2.488-13.219)	0.000*
M1c	3.784	(0.984-14.560)	0.053	2.945	(1.110-7.811)	0.030*	0.94	(0.492-1.796)	0.85
PKM2 expression									
Low	Ref			Ref			Ref		
High	6.402	(2.107-19.453)	0.001*	1.873	(0.898-3.906)	0.094	2.545	(1.325-4.888)	0.005*

Cox regression and Logistic regression analysis were used to differentiate the prognostic markers; HR, hazard ratio; OR, odds ratio; CI, Confidence interval; *Significance defined by p<0.05.

differentiation (HR: 2.616, 95% CI: 1.424–4.807; P = 0.002) were the most potent independent predictors of OS. Detailed results of the univariate and multivariate analyses of ORR, PFS, and OS are presented in Table 3.

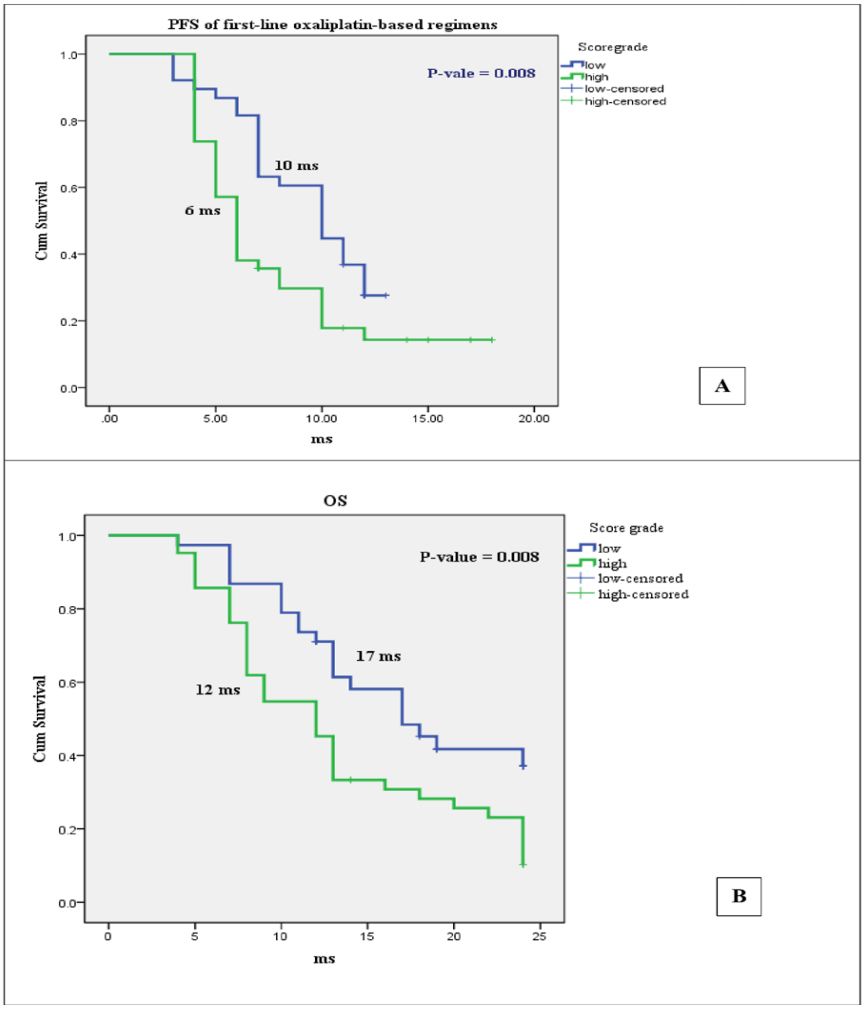

Figure 3. Patient's Outcome According to *PKM2 IHC* Expression: A) PFS, B) OS.

Discussion

PKM2 is prominently expressed in cancer cells and is recognized for its key role in tumorigenesis [23]. *PKM2* is essential in sustaining the metabolic process of cancer cells [24]. *PKM2* overexpression has been correlated with drug resistance and cancer progression, highlighting its potential as a valuable therapeutic target in oncology [25].

In our study, slightly more than half (52.2%) of the pathologically confirmed metastatic CRC cases showed high *PKM2* expression based on IHC analysis. These findings align with a sub-analysis performed by Cui R et al, which found a significant correlation between TNM stage and *PKM2* protein expression (P = 0.003), with elevated *PKM2* expression observed in 79.2 % of stage IV CRC patients [22]. Moreover, Zhou et al. [26] have demonstrated that *PKM2* overexpression was associated with a more advanced tumor stage (P = 0.038) and was observed in 93.3% of metastatic cases, a proportion higher than that found in our study

The current study identified a statistically significant association between high *PKM2* expression and younger age median (P = 0.001). In contrast, studies [22, 26, 27] reported no significant link between age and *PKM2* expression, which may be attributed to variations in study populations, sample sizes, and different age cutoff values in patient stratification.

Our study revealed a significant association between elevated *PKM2* expression and RAS mutations (P = 0.013), while no significant association was observed with BRAF mutations. This result mirrors the findings of Sfakianaki et al., who reported a strong correlation between elevated *PKM2* mRNA expression and KRAS mutations (P = 0.009), but only a modest association with BRAF mutations (P = 0.041) [27]. The discrepancy between our results and theirs regarding BRAF may be attributed to differences in cohort size or population characteristics, underscoring the need for further validation in larger, more diverse populations.

The observed link between *PKM2* and RAS mutations supports the hypothesis that *PKM2* contributes to RAS-driven CRC progression. This is consistent with earlier mechanistic studies indicating that RAS mutations activate the mitogen-activated protein kinase (MAPK)/ extracellular signal-regulated kinase (ERK) signaling cascade, enhancing *PKM2* activity. Notably, Yang et al. [28] demonstrated that ERK2 phosphorylates *PKM2*, promoting its nuclear translocation and enabling transcriptional activation of genes involved in cancer metabolism and proliferation

Recent experimental studies have further elucidated *PKM2*'s role in oncogenic signaling. Chen et al. [29] showed that the protein Melanocyte proliferating gene 1 (MYG1) upregulates *PKM2* through the adenosine monophosphate-activated protein kinase (AMPK)/ mechanistic target of rapamycin (mTOR) pathway, enhancing glycolytic flux and promoting CRC cell growth [29]. Similarly, Yu et al. reported that Copine 7 (CPNE7) interacts with *PKM2* and stimulates the MAPK pathway, forming a positive feedback loop that drives tumor aggressiveness [30]. Together, these studies support our findings and show that *PKM2* is closely linked to RAS signaling and tumor progression in CRC.

Additionally, our findings suggest a potential link between *PKM2* expression and mucinous differentiation in CRC, although the association was borderline significant (P = 0.05), it raises intriguing questions. Mucinous CRCs are known to depend heavily on glycolysis to meet their biosynthetic demands, particularly for mucin production. As a central regulator of the Warburg effect, *PKM2* may facilitate this metabolic shift by promoting the accumulation of glycolytic intermediates essential for mucin biosynthesis. Supporting this, experimental studies in CRC cell lines have shown that silencing *PKM2* leads to decreased glucose uptake and lactate production, impairing glycolysis and sensitizing cells to chemotherapy [19].

Moreover, the frequent co-occurrence of KRAS mutations in mucinous CRCs may further explain the link. As mentioned earlier, KRAS mutations activate the MAPK/ERK pathway. This pathway, in turn, promotes ERK-mediated phosphorylation and nuclear translocation of *PKM2*, linking oncogenic signaling to metabolic reprogramming [31]. These findings collectively provide a mechanistic rationale for the observed association between *PKM2* expression and mucinous histology. However, it is important to note that a previous study [22] did not find a significant correlation between *PKM2* and mucinous features, possibly due to differences in methodology, sample sizes, or classification criteria for mucinous differentiation.

In the current study, we evaluated the relationship between *PKM2* expression and the response of oxaliplatin-based chemotherapy and survival, we observed that there was a statistically significant lower PFS (6 ms, 95% CI: 5.22–6.77 ms) in high *PKM2 IHC* expression than in those with low *PKM2 IHC* expression tumors (10 ms, 95% CI: 7.99–12.003 ms; P = 0.008). These results are compatible with those reported by Sfakianaki et al. in their study of metastatic CRC patients, treated with FOLFOX, which demonstrated that elevated *PKM2* mRNA expression was significantly associated with reduced PFS (6.7 ms, 95% CI: 4.8–7.5) versus (9.1 ms, 95% CI: 7.7–11.2) in patients with lower expression levels, P = 0.003 [27].

In addition, our study demonstrated a significantly shorter median OS in patients with high *PKM2 IHC* expression (12 ms, 95% CI: 9.36-14.63), in contrast to (17 ms, 95% CI: 11.82-22.17) observed in patients with low *PKM2* expression, P = 0.008. These data were in line with Sfakianaki et al.'s study, who also reported a notable association between high *PKM2* mRNA expression and shorter OS (21.9 months, 95% CI: 16.0–24.7) compared to low expression (30.2 months, 95% CI: 24.0–37.3; P = 0.004) [27]. In addition, Cui et al reported comparable results as higher *PKM2* protein expression levels were significantly linked to poorer OS in CRC patients (P < 0.0001) [22].

Finally, the current study demonstrated that *PKM2* expression, evaluated by IHC, could be used as an independent predictor factor for OS, as shown in the multivariate analysis (HR: 2.545, 95% CI: 1.325–4.888; P = 0.005). These findings are consistent with the findings of the Sfakianaki et al. study, which reported that high *PKM2* mRNA expression independently predicted lower OS (HR: 1.94, 95% CI: 1.38–3.32; P = 0.001) in their multivariate analysis [27]. Furthermore, another study reported that *PKM2* expression was one of the independent predictor factors for OS in patients with CRC (HR: 3.712, 95% CI: 2.233-6.173; P = 0.000) [22], which agrees with our results.

In conclusion, our findings revealed that elevated *PKM2* expression, as determined by IHC, was associated with lower ORR, PFS, and OS in metastatic CRC patients treated with oxaliplatin-based regimens as the first-line treatment. Moreover, *PKM2 IHC* expression may serve as an independent prognostic factor in metastatic CRC, suggesting that *PKM2* may be a promising target for therapeutic approaches in CRC and other malignancies.

This study has several limitations, including a small sample size, a relatively short duration of follow-up, and limited resources to use anti-EGFR and anti-VEGF. Further large-scale, multicenter prospective studies are required to validate our findings regarding oxaliplatin resistance and survival outcomes in metastatic CRC.

Author Contribution Statement

All authors were involved in planning the research and study design. Ahmed Refaat and Nada Mohammad Hussien were responsible for analyzing and interpreting clinicopathological data, treament responses, and patient survival outcomes. Marwa T. Hussien conducted the histopathological evaluation of the colorectal tumor samples. Abeer Ibrahim and Rabab Mohamed Mumdouh Farghaly played roles in drafting the manuscript. All authors reviewed and approved the final version of the manuscript.

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Approval

This study was submitted in partial fulfillment of the requirements for the M.D. degree in Medical Oncology by Nada Mohammad Hussien, Assistant Lecturer of Medical Oncology, South Egypt Cancer Institute, Assiut University.

Ethical Approval

Regarding ethical considerations, this study was approved by the Institutional Ethical Committee of South Egypt Cancer Institute, and its Institutional Review Board (IRB) approval number is IORG0006563-602. The drugs were supplied either by treatment decisions at state expense or by health insurance.

Data Availability

All data generated and analyzed during this study can be accessed through direct communication with the corresponding author and the agreement of all research team members.

Study Registration

This study was not a clinical trial, guideline, or meta-analysis and was therefore not registered in any clinical trial or systematic review registry.

Conflict of Interest

We have no conflicts of interest to disclose

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