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Study the Gene Expression of *PIK3CA* in Tissue and Blood Samples of Iraqi Patients with Colorectal Cancer

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Abstract

Objectives: The primary objective of this investigation is to explore the correlation between gene expression of *PIKCA3* and colorectal cancer in both tissue and blood samples, while also performing a comparative analysis between these sample types.
Methods: This research involved the collection of blood and tissue samples. The study was conducted at the Gastroenterology Hospital within the Medical City Department in Baghdad, Iraq, spanning from December 2021 to December 2022. A total of 80 volunteers, encompassing both genders and aged between 24 and 77, participated in the study. The real time qRT-PCR analysis method was employed to assess the expression of *PIKCA3* genes within patient and control groups.
Results: The findings indicate significant distinctions in Ct *PIK3CA* between blood and tissue samples across all study groups. Moreover, *PIK3CA* expression displayed a notable association with CRC patients, showing higher expression levels compared to the other groups. This disparity was particularly pronounced when comparing tissue and blood samples. Statistical analysis, specifically Receiver Operating Characteristic (ROC) Curve Analysis, affirmed the excellent predictive capacity of the AUC (Area Under the Curve) value. The study established that *PIK3CA* gene expression in tissue outperformed that in blood, demonstrating a considerably elevated gene expression in tissue ($P < 0.001$). Notably, the fold increase in gene expression within CRC patients was significantly higher compared to the group of healthy participants, with values of 23.630 in blood samples and 124.810 in tissue samples, respectively. The present study underscores the prevalence of *PIK3CA* gene mutations in colorectal carcinomas.
Conclusion: The frequent occurrence of *PIK3CA* gene mutations in colorectal carcinomas may hold promise for the development of targeted therapy combinations. These could involve inhibiting both the PI3K kinase itself and addressing associated pathway anomalies.
Keywords: *PIKCA3*, gene expression, qRT-PCR, colorectal neoplasms

Introduction

Colorectal cancer occurs more frequently than any other type of digestive cancer. It is projected to have a higher fatality rate than cardiovascular disease. It accounts for the vast majority of fatal cancer cases.[1-3] It is the fourth most common kind of cancer in Iraq and the third most common in the globe.[4] While CRC is a major issue in the West, it is also a significant health concern in Iraq, where it ranks third in terms of tumor frequency (excluding non-melanoma skin cancer) among Iraqi citizens of both sexes.[5-7]

Both hereditary and environmental factors contribute to CRC's pathogenesis. Gene-environment interaction, which involves the interplay of genetic variations and environmental risk factors, has been hypothesized to enhance colon, rectal, and bladder cancer risk.[8] Deficits in vitamin C and D have been identified as key risk factors among Iraqi CRC patients.[4] Biomarkers have been the subject of much study in an effort to better diagnose patients and forecast how they will respond to treatment. Expert panels have only recommended a small subset of biomarkers thus far. The discovery of new prognostic indicators has the potential to lead to improved CRC management guidelines, which in turn could increase survival rates.[9,2]

Typically, CRC develops from a benign expansion of mucosal epithelial cells. Polyps are slow-growing growths that might cause problems for 10–20 years before they become malignant. Cellular alterations that increase susceptibility to developing into cancer in the colon or rectum are known as precancerous conditions. These diseases do not appear to be cancerous at this time. However, if these abnormalities are left untreated, they can progress to colorectal cancer.[10] Different miRNA expression patterns were observed between patients with colorectal cancer (CRC) and healthy controls; miRNAs have a role in tumor progression and metastasis.[11]

The phosphatidylinositol 4,5-bisphosphate 3-kinase (PI3K) alpha catalytic subunit gene *(PIK3CA*) is frequently mutated in cancer helps cells live longer and divide more frequently.[12] Numerous investigations have shown that CRC is just one type of malignant tumor that contains activated *PIK3CA* mutations.[13] PIK3s are thought to play a role in the molecular pathogenesis of *PIK3CA* mutations, which may stimulate AKT signaling and ultimately promote cancer cell proliferation and migration by activating the p110a enzyme, the major catalytic component of PI3K.[14] Identification of individuals with *PIK3CA* mutant CRC will benefit from a thorough comprehension of the impact of *PIK3CA* mutations on the clinicopathological and molecular features of the disease. This will help clarify the significance of *PIK3CA* mutations in CRC development.[15] This study intends to compare and contrast *PIKCA3* gene expression in tissue and blood as a possible indicator of CRC.

Materials and Methods

This study's actual activity, including collecting blood and tissue samples, lasted a full year, from December 2021 to

December 2022. The Gastroenterology Hospital at the Medical City Department provided the study's 80 volunteers, both male and female, ranging in age from 24 to 77.

Blood samples were taken for molecular testing from each patient, and endoscopic colonic biopsies or surgical resections were used to diagnosis each case at the aforementioned institution. According to the information obtained from the hospital's histopathology report, the samples were separated into three groups: Group 1: Control groups this group typically consists of twenty control people 9 males (tissue) and eleven females (blood) of different ages and genders. Group 2: Colitis group nineteen of individuals in the colon and rectum with colitis, male and female, nine males (six blood and three tissue) and ten females (seven blood; three tissue), so it was considered a positive control. Group 3: Malignant group's forty-one, 21 male (eight tissue, thirteen blood) and 20 female (twelve tissue, eight blood) this group with colon and rectum adenocarcinoma approved in the study of various ages and sexes. Both the control and patient groups had about 250 µl of peripheral whole blood drawn and added to 750 µl of TRIzol® LS Reagent in Eppendrof's tubes. The ratio was (3 TRIzol: 1 blood). (Trizol LS Reagent, 2012). (Tissue sample) Tissue Ruptor transfer quickly into a precooled mortar with liquid nitrogen. Homogenizer Grind thoroughly into powder. Use more liquid nitrogen if needed. Incomplete grind can affect RNA yield and quality. The materials were stored at −23°C (RNA Extraction procedure by means of TransZol Up Plus RNA kit) until they could be used in a molecular analysis. Real Time qRT-PCR analysis method was used to investigate *PIKCA3* genes expression in patients and control groups.[16]

Statistical Analyses

SPSS for Windows, version 22 (SPSS Inc., Chicago, Illinois, United States) was utilized to analyze the data statistically. The information was presented with a mean and standard deviation (SD), Receiver Operating Characteristics (ROC) analysis. Using a Shapiro–Wilk normality test, it was determined if the examined parameters followed a Gaussian distribution. One Way Analysis of Variance (ANOVA): statistical test for the difference between k independent groups with using Hochberg GT2 and Games-Howell test. Level of *P* value as: Not significant $P > 0.05$, Significant $P < 0.05$.

Results and Discussion

The results demonstrated that Ct *PIK3CA* blood and tissue samples from all study groups showed statistically significant variations Table 1.

These data shown in Table 1 and Figures 1 and 2 demonstrated a higher level of *PIK3CA* expression in CRC patients compared to controls, both in tissues and blood samples. Research on colorectal cancer patients has revealed that *PIK3CA* exon 20 mutations may serve as a marker for resistance to anti-EGFR therapy.[17] According to the findings of Smeby et al.[18] The significance of genetic variations may be context-dependent; hence, the relationship of *PIK3CA* mutations with clinicopathologic features may be context-dependent.

In the present study, there were significant ($P \leq 0.05$) differences between Ct *PIK3CA* Blood and tissue samples in all study groups, as shown in Table 2 the results of current study

Table 1. **Descriptive and statistical test of Ct *PIK3CA* among study groups and site**

Site	Study groups		Ct *PIK3CA*		
			R	*P* value	
Blood	Colitis	Ct GAPDH	0.053	0.007	**0.003***
	Tumor		0.014	0.005	
	Control		0.297	0.375	
Tissue	Colitis		0.041	0.070	
	Tumor		0.063	0.006	
	Control		0.043	0.912	

*means significant at ($P \leq 0.05$), Ct GAPDH: Ct House Keeping Gene, R: Pearson correlation.

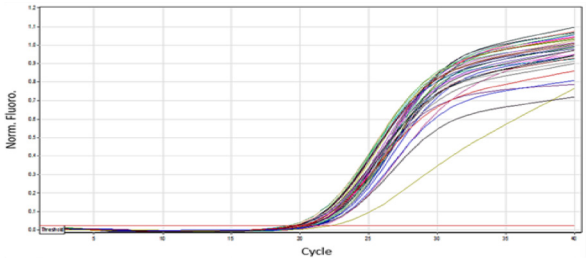

Fig. 1 ***PIK3CA* amplification plots by qPCR. Samples included all study groups. Ct values ranged from 24.61–29.07. The photograph was taken directly from Qiagen (Rotor gene 6000) qPCR machine.**

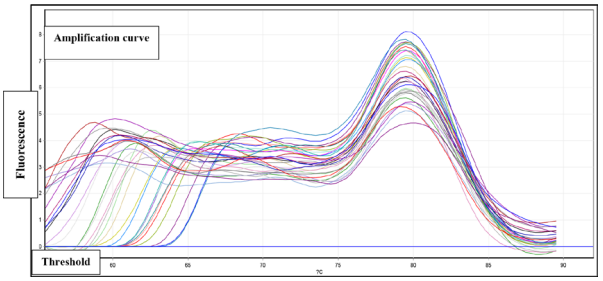

Fig. 2 ***PIK3CA* dissociation curves by qPCR samples included all study groups. Melting temperature ranged from 78°C to 81°C, no primer dimer could be seen. The photograph was taken directly from Qiagen (Rotor gene 6000) qPCR machine.**

Table 2. **Descriptive and statistical test of Ct *PIK3CA* among study group and site**

Site		Study group			F	*P* value
		Colitis	Tumor	Control		
Ct *PIK3CA* Blood	Mean	22.760	25.689	22.714	7.356	**0.002***
	± SD	2.996	0.533	2.600		
	Min.	19.150	24.610	19.910		
	Max.	26.390	28.860	25.650		
Ct *PIK3CA* Tissue	Mean	20.536	27.120	20.627	160.065	**0.000***
	± SD	0.848	0.973	0.975		
	Min.	19.550	26.120	19.760		
	Max.	22.310	29.070	22.310		
T test		3.268	4.185	2.533		
P value		**0.003***	**0.001***	**0.022***		

*means significant at ($P \leq 0.05$).

demonstrated that *PIK3CA* expression were associated with CRC patients (highly expressed) than those in other groups, especially in tissues compared with blood samples. a quantitative RT-PCR test was utilized to assess *PIK3CA* mRNA expression and compare it between the tumor, colitis, and control groups after normalization of the *PIK3CA* gene's Ct (cycle threshold) values. Further, gene expression fold change was calculated using the relative quantification equation ΔCt (control) = CT (gene) - CT(HKG),

ΔCt (patient) = CT (gene) - CT(HKG). The expression ratio was calculated according to the formula: $2^{-\Delta CT}$ = Normalized expression ratio, ΔΔCt = ΔCt (patient) - ΔCt (control)

Finally, Fold change = $2^{-\Delta\Delta Ct}$ Normalized expression ratio.[19]

This study confirmed the findings of Zhang et al.,[20] which found that *PIK3CA* exon 20 mutations occur in 2%–18% of metastatic CRCs, the prevalence of *PIK3CA* mutation in this analysis was 3.5%, which is in line with previous Asian studies. In light of the findings by Juan et al.[21] that overall or exon-specific *PIK3CA* mutations were not associated with key clinicopathological parameters, such as tumor differentiation and disease stage and identified a strong correlation between *PIK3CA* mutations and KRAS mutations, the nature of their relationship warrants further investigation. Due to mutations in exons 20 and 9 of *PIK3CA*.

PIK3CA mutations and programmed death ligand-1 (PD-L1) expression were found in 21.4% and 10.3% of CRC patients, respectively, according to Ahn et al.[22] *PIK3CA* mutations were connected with favorable prognostic variables, prolonged relapse-free survival, and expression of PD-L1. A significant correlation was found between *PIK3CA* mutations and cancer of the right colon ($P = 0.011$), and they were correlated inversely with lymph node metastases ($P = 0.026$). Additional *PIK3CA* mutations are a positive prognostic factor, and the fact that they agreed with these findings suggested that the tumor had greater *PIK3CA* expression rates in CRC than those in other groups, particularly in tissues in comparison to blood samples.

According to Li et al.[23] the PI3K signaling pathway is essential for the migration, growth, metastasis, and survival of tumor cells. A wide variety of tumor forms have been found to include widespread *PIK3CA* gene mutations, according to multiple lines of evidence. *PIK3CA* is implicated in both the progression of tumors and the development of resistance to many drugs, thereby influencing overall survival. While a few studies have reported *PIK3CA* mutations in Right Colon Cancer.

The results demonstrated that the ΔCT (normalization Ct values) for each group of studies was significantly ($P \leq 0.05$) higher in the patient group compared to the other groups. 8.145 ± 0.539 in blood samples and 10.133 ± 0.906 in tissue compared to other groups, and it became clear that tissue gene expression is higher than blood samples in tumor patients. In the colitis group, the mean gene expression in blood samples was 5.748 ± 2.445 and in tissue samples it was 3.565 ± 0.783. In the control group, the mean gene expression in blood samples was 5.736 ± 2.959 and in tissue samples it was 3.349 ± 0.776. In addition, as shown in Table 3, there were significant differences between ΔCT blood and tissue samples in all study groups.

Table 3. **Descriptive and statistical test of ΔCT among site and study groups.**

Site	ΔCT	Study groups			F	*P* value
		Colitis	Tumor	Control		
Blood	Mean	5.748	8.145	5.736	5.258*	**0.011***
	± SD	2.445	0.539	2.959		
	Min.	2.350	7.240	2.680		
	Max.	9.320	8.920	12.360		
Tissue	Mean	3.565	10.133	3.349	220.408*	**0.000***
	± SD	0.783	0.906	0.776		
	Min.	2.750	8.660	2.220		
	Max.	4.580	11.400	4.960		
T test		2.911*	5.794*	3.571*		
P value		**0.010***	**0.000***	**0.002***		

***means significant at ($P \leq 0.05$).

The Receiver Operating Characteristic Curve Analysis of Studied Parameters

The receiver operating characteristic curve analysis used for the evaluation of the diagnostic value of *PIK3CA* gene expression in tissue and blood among colorectal cancer

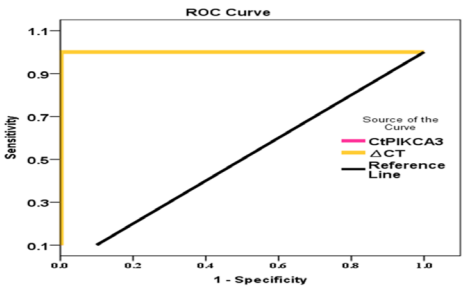

Fig 3. **Receiver Operating Characteristics (ROC) curves to comparison ct and ΔCT *PIKCA3* gene expression in tissue and blood level among CRC patients.**

Table 4. **Statistical analysis of Receiver Operating Characteristics (ROC) to comparison ct and ΔCT *PIKCA3* gene expression in tissue and blood level among CRC patients**

Parameter	Area Under the Curve				
	AUC Area	AUC Explanation	AUC *P* value	%Sensitivity	%Specificity
Ct *PIKCA3* value comparison between tissue and blood among CRC patients	1.000	Excellent	.000**	100	95.2
ΔCT *PIKCA3* value comparison between tissue and blood among CRC patients	1.000	Excellent	.000**	100	95.2

AUC: Area Under the Curve, **means significant at 0.05 and 0.01 levels, respectively.

Table 5. **Descriptive and statistical test of ΔΔCT among site and study groups**

Site	ΔΔCT	Study groups			F	*P* value
		Colitis	Tumor	Control		
Blood	Min.	−6.252	−5.922	−1.362	6.062*	**0.006***
	Max.	0.628	3.758	0.318		
	Mean	−2.861	−2.866	−0.290		
	± SD	2.434	2.959	0.008		
Tissue	Min.	−7.383	−7.913	−1.473	220.408*	**0.000***
	Max.	−5.553	−5.173	1.267		
	Mean	−6.568	−6.785	0.000		
	± SD	0.783	0.776	0.906		
T test		4.963	5.861	0.906		
P value		**0.000***	**0.000***	0.377		

** means significant at (P ≤ 0.05) and (P ≤ 0.001) levels respectively.*

Table 6. **Descriptive and statistical test of fold $2^{-\Delta\Delta Ct}$ among site and study groups**

Site	Fold $2^{-\Delta\Delta Ct}$	Study groups		
		Colitis	Tumor	Control
Blood	Min.	0.647	0.074	0.802
	Max.	76.205	60.624	2.570
	Mean	20.630	23.630	1.300
	± SD	26.054	22.949	0.521
Tissue	Min.	0.042	36.085	0.416
	Max.	159.05	241.07	2.777
	Mean	70.950	124.81	1.186
	± SD	65.990	59.356	0.744

patients shown in Table 4 and Figure 3. An excellent prediction of AUC value results was seen for *PIK3CA* gene expression in tissue compared to *PIK3CA* gene expression in blood, were found to be associated with a significantly increased gene expression in tissue ($P < 0.001$) and ($P < 0.05$), respectively, patients which indicated as markers, especially for *PIK3CA* gene expression in tissue, to discriminate expression in blood patients. While the sensitivity and specificity at 100%, 95.2% respectively.

The results of ΔΔCT are illustrated in Table 5. The results shows that there was a significant ($P \leq 0.05$) in blood samples, colitis and tumor as compared with control groups. Further, there was significant difference in tissue samples in colitis and tumor as compared with control groups.

The $2^{-\Delta\Delta Ct}$ values have been used by each research group to determine the *PIK3CA* gene's expression. Every group's $2^{-\Delta\Delta Ct}$ results have been compared to the control group. these findings imply that the mean of $2^{-\Delta\Delta Ct}$ differed significantly ($P \leq 0.05$) among these groups (Table 6). In carcinomas of the colon, breast, brain, liver, stomach, lung, ovary, and head and neck, somatic mutations of *PIK3CA* have been reported by Akagi et al.[24] Mutated *PIK3CA* is capable of activating Akt in the absence of growth factors. Sood et al.[25] discovered that in patients with KRAS wild-type colorectal cancers, maintaining PTEN expression and *PIK3CA* wild-type status was associated with enhanced overall survival compared to *PIK3CA* mutant tumors. More than 80% of *PIK3CA* mutations in human malignancies are found in these three codons, according to data from the Catalogue of Somatic Mutations in Cancer (COSMIC) database. Overexpression of *PIK3CA* in mice models of breast cancer has been demonstrated to result in tumorigenesis, and *PIK3CA* mutations have been linked to activation of downstream PI3K/Akt/mTOR signaling as well as transforming effects in cell culture. They also have ramifications for treatment and prognosis.[26] Further, breast cancer tissue was discovered to express much higher levels of *PIK3CA* than normal breast tissue by Palimaru et al.[27] Consistent with our findings, a hyperactive PI3K pathway was found to be the outcome of elevated *PIK3CA* expression.

Values of $2^{-\Delta\Delta Ct}$ were compared between groups, with the group of apparently healthy participants used as a reference to determine fold changes in gene expression relative to housekeeping genes. The fold of gene expression in the CRC patients was higher upregulated (increased) than healthy participant group. 23.630 in blood samples, and 124.810 in tissue samples respectively, fold number in colitis (positive control group) was 20.630 in blood samples, and 70.950, in tissue samples respectively, while the fold number in control was 1.300 in blood samples, and 1.186, in tissue samples (Table 5) displays the outcomes. Every group's $2^{-\Delta\Delta Ct}$ results have been compared to the control group. The relative gene expression is usually set to 1.186,1.300 for reference samples because Δ*ΔCT* is equal to

0 and therefore 2^0 is equal to 1.[28] Based on quantitative RT-PCR analysis, Akagi et al.[24] found that the mean mRNA level of *PIK3CA* in ESCC tissues was 2.61-fold greater than that in those with non-tumorous esophageal epithelia ($P \leq 0.001$). In addition, this is the first paper to provide such a thorough examination of *PIK3CA* expression in ESCC. These findings suggest a potential function for *PIK3CA* in ESCC progression and as an indication of lymph node metastasis. This study findings that CRC patients had greater gene expression levels compared to healthy controls are supported by these data.

Changes in the PI3K enzyme family are observed in human cancer, and mutations in the *PIK3CA* gene have been uncovered in several types of cancer, including colorectal cancer (CRC). In addition to this, the *PIK3CA* mutation is associated with a substantial reduction in the patient's chance of surviving the disease. Different biological effects and the promotion of carcinogenesis are both caused by mutations in *PIK3CA* (exon 9 and exon 20), which are responsible for the mutations.[29]

According to Wang and Pan et al.[30] patients with colorectal cancer frequently exhibit aberrant gene expression and abnormal signaling pathways. However, the mutant patterns of *BRAF*, *KRAS*, and *PIK3CA* were not connected to the general or clinicopathological aspects of the patients. The mutant pattern has the potential to serve as an independent determinant in determining the prognosis of colorectal cancer.

Mutations in tumor suppressor genes such APC, TP53, and SMAD4, as well as the oncogene *KRAS*, are common in colorectal cancers. This is one of the colorectal malignancies' distinguishing characteristics.[31]

These mutations contribute to the development of cancer by causing a dysregulation of important pathways of cancer signaling, including those that are activated when receptor tyrosine kinases are activated.[32]

From the present study, it concluded that Colorectal carcinomas are characterized by frequent mutations of the *PIK3CA* gene, which may present a window of opportunity for the development of targeted therapeutic combinations that inhibit both the PI3K kinase and related pathway abnormalities.

The limitations of the present study include it's had a specific time, information collected from a single hospital, and cases are not readily available that can be studied., larger sample sizes and diverse methodologies may be the focus of further studies.

Conclusion

We found significantly increased *PIK3CA* expression in colorectal adenocarcinoma tissue compared to normal colorectal tissue. We also found increased expression in colorectal adenocarcinoma tissue more than blood. So, frequent mutations of *PIK3CA* gene in colorectal carcinomas may represent an opportunity for targeted therapy combination development inhibiting both the PI3K kinase itself and associated pathway defects. As for recommendations, it is possible to recommend the use of Western blot technology to measure the level of proteins for the studied gene in order to clarify their role more accurately.

Acknowledgments

None declared by the authors.

Ethical Permissions

The research was ethically approved by the Ethics Committee of the Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq. Committee number dated CSEC/1121/0076. Written informed consent was obtained from all patients.

Conflicts of Interests

No conflicts of interests were reported.

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