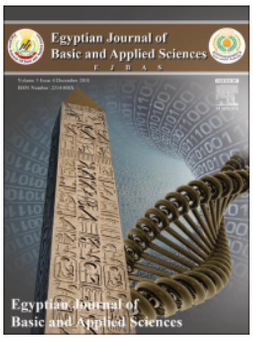

Egyptian Journal of Basic and Applied Sciences

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/teba20

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To cite this article: Ameer Ali Imarah, Rana Ahmed Najm, Haider Ali Alnaji, Saleem Khteer Al-Hadraawy, Abbas F. Almulla & Hussein Raof Al-Gazali (2023) Investigate the role of *PIK3CA* gene expression in colorectal polyp development, Egyptian Journal of Basic and Applied Sciences, 10:1, 594-604, DOI: 10.1080/2314808X.2023.2245992

To link to this article: https://doi.org/10.1080/2314808X.2023.2245992

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Published online: 21 Aug 2023.

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EGYPTIAN JOURNAL OF BASIC AND APPLIED SCIENCES
2023, VOL. 10, NO. 1, 594–604
https://doi.org/10.1080/2314808X.2023.2245992

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Investigate the role of *PIK3CA* gene expression in colorectal polyp development

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ABSTRACT

Background: Polyps are pathological concerning histological changes that develop during colorectal and rectal cancer. The degree of polyp severity is due to their ability to transform the polyp cells from normal to malignant. PIK3CA is a gene that plays an important role in cell cycle regulation.
Objective: The study aims to evaluate PIK3CA gene expression in tissues of colorectal polyps.
Methods: Two study groups employed in the current study were patients ($n = 68$) and the control group ($n = 50$). Immunohistochemical techniques were applied to paraffin-embedded tissues to localize PIK3CA expression.
Results: The current study showed a significant difference between the control and patients group in PIK staining results; in the control group, there was no case positive for PIK, while in the patient group, all cases were positive. In the rectum, there was no significant difference between cases given positive and negative. Among histological types, according to tubular adenoma histological type, in the right colon, the cases which gave positive for PIK were significantly lower than negative, while in the left colon, the cases which gave positive were significantly higher than negative.
Conclusion: The PIK3CA gene initiates polyp formation and benign tumoral growths, which may subsequently undergo malignant transformation.

ARTICLE HISTORY
Received 21 January 2023
Revised 30 July 2023
Accepted 1 August 2023

KEYWORDS
Colorectal polyp; colorectal tumor; *PIK3CA*; IHC

Introduction

A polyp is a tumor that protrudes into a hollow vessel anywhere in the genitourinary, gastrointestinal, or respiratory tracts. The polyps usually arise from the inner layer of the organ (mucosal layer), even though a few submucosal (second inner layer) disorders can lead to mucosal growth into the lumen and protrusions that resemble mucosal polyps. Not all polyps necessarily display malignant characteristics [1]. Colorectal polyps are classified histologically as neoplastic such as Hyperplastic polyps, Lymphoid aggregates, Hamartomas, and Inflammatory polyps, or nonneoplastic, such as Tubular adenomas, Tubulovillous adenomas, and Villous adenoma [2]. Tissues in these polyps included 0–25%, 25–75%, and 75–100% of villous tissues, respectively [3]. Most polyps found during screening are noncancerous and harmless, but they can be the underlying source of

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uncomfortable gastrointestinal symptoms like bleeding, diarrhea, and constipation [4]. The malignant characteristics and following screening intervals vary based on the type of polyp; nevertheless, the adenoma-carcinoma sequence has historically been described as a homogeneous progression from normal mucosa, to adenoma (benign tumor), to carcinoma (malignant tumor), via an underlying homogenous carcinogenic route [5,6]. One of the most important polyps is the neoplastic polyp due to their malignant potential, the earliest stage in the development of colorectal cancer. Due to previous causes, it is very important to diagnose and treat these polyps appropriately early; when a simple office treatment is necessary to remove them, colorectal cancer development can be stopped, and suffering and death can be avoided [7].

When colonic adenomas grow larger than 2 centimeters in diameter, they can be a precursor to colorectal adenocarcinoma, making them therapeutically significant [8]. The severity of this type of polyps came from the ability to cumulate mutations associated with cell cycle control over years [1] and *PIK3CA*, one of these genes [9]. The phosphatidylinositol 3-kinases (PI3Ks) are involved in signaling pathways important for tumorigenesis, including those involved in apoptosis, proliferation, and migration [10]. Phosphoinositide-3-kinase, catalytic alpha polypeptide (*PIK3CA*), is an integral member of the lipid kinase family, encoding the p110 catalytic subunit of the kinase [11]. *PIK3CA* gene is located on chromosome 3 long arm (3q26.32) and is involved in cell control via expression to catalytic subunit p110 in phosphatidylinositol 3 Kinase. Phosphoinositide 3 The kinase enzyme interacts with phosphatidylinositol-3-phosphate, a membrane component, to catalyze the phosphorylation of AKT, which then activates the signaling pathway. Because the activation of this pathway depends on outside signals like growth factors, this gene is classified as a proto-oncogene [12].

PIK3CA gene has two mutations in Exons 9 and 20, which affect the helical and catalytic domains. Activating point mutations in these kinase activity-associated areas rise, activate the alpha serine/threonine-protein kinase (AKT), and cause carcinogenesis by decreasing apoptosis, contact inhibition, and tumor expansion into surrounding normal tissues [12]. PI3K is involved in many functions inside cells, such as cell growth, division, and movement, and may participate in the maturation of adipocytes [13]. Therefore, the current study aimed to evaluate *PIK3CA* gene expression in tissues of colorectal polyps.

Materials and methods

Sixty-eight individuals clinically diagnosed with colorectal polyp and 50 subjects with colitis as a control group were implemented in the current study between November 2021 and October 2022. Their paraffin-embedded tissues were collected from the histopathology unit in AL-Sader Medical City, AL-Najaf province, Iraq, after ethical approval from the medical ethics committee at the University of Kufa, Iraq. Hematoxylin and eosin (H&E) staining and immunohistochemistry for PIK antibodies were implemented. All patients without case histories were excluded.

Immunohistochemical assay

PIK3CA expression in tissues of colorectal polyp evaluated by Immunohistochemistry technique (IHC) using Rabbit anti-human PI3 Kinase p110a provided by MyBioSource/USA in dilution factor 1:100-1-200. The IHC procedure was done as mentioned in a study conducted by [14] the intensity of staining was recorded as 0, 1, 2, and 3, meaning no stain, weak, moderate, and strong, respectively [15]. Stained cells evaluated as a proportion (%) as

0-<10% of cells stained, which gives a score of 0, 10–25% of cells indicate score 1, 26–50% of cells stained given score 2, 51–75% of cells stained with score three and when cells stained more than 75% of cells in the field give score 4. Score index = score *intensity. If the score index is more than 10, meaning positive, while if the score index is less than 10, meaning negative. Positive and negative control were included with each run. Demographic characteristics of patients with polyps were collected and analyzed, including age and sex. The type and location of the polyp were analyzed as clinical characteristics.

Statistical analysis

Graph Pad Prism 8 software is used to perform all statistics of the current study. A T-test was used to compare the results of patients and the control group. Spearman's correlation was used to show if there was any correlation between the results. The significant level is < 0.05 [16,17].

Results

The present study included 68 subjects with polyps as a patient's group and 50 subjects healthy as controls. In the patient's group, 35 subjects were males, and 33 were females (Figure 1), while in the control group, 25 subjects were males and 25 were females. There was no significant difference between males and females. According to age, the patient group was divided into three age groups (50–59 years), (60–69 years), and (70–80 years), including 37 patients, 7 patients, and 24 patients, respectively, as shown in Table 1.

Colorectal polyp, according to the site

The presence of polyps is divided as follows, right colon 27, left colon 26, and 15 cases were in the rectal area, as illustrated in Figure 2. There is no difference in the incidence of polyps between the right colon area and the left area. In contrast, the appearance of polyps in the rectal area of the lower GIT system is less common than in other sites of the colon.

Positive and negative PIK staining according to the site of the polyp

Negative IHC staining for PIK was observed in tissues from control groups, while variable positive PIK staining was found in tissues from patients. The number of cases positive for PIK staining in the left colon was higher than in the rectum, and the cases positive in the rectum were higher than in the right colon (Figure 3). A comparison of positive and negative PIK staining in the different areas indicates that negative cases were higher than positive in

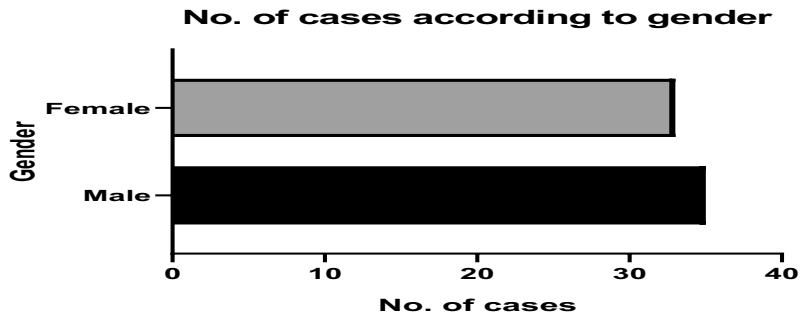

Figure 1. Distribution of cases according to gender.

Table 1. Distribution of patients according to age group.

Age (years)	Observed No.	Expected No.	Residual
50–60	37	22.7	14.3
60–70	7	22.7	−15.7
70–80	24	22.7	1.3
Total	68		

the right colon. In comparison, in the left colon, the number of positive cases was higher than negative (Figures 3 and 4). It should be noted that the number of cases given positive is relatively equal to cases given negative.

Distribution of cases according to histological types

The majority of histological types in this study was tubular adenoma ($n = 53$), and only 15 cases were tubulovillous adenoma (Figure 5). Histological type-tubular adenoma and results of PIK staining among the site of the polyp are shown in Figure 6. In the right colon, the number of cases with tubular adenoma given negative for PIK staining was significantly higher than those given positive. The positive cases were significantly higher in the left colon than

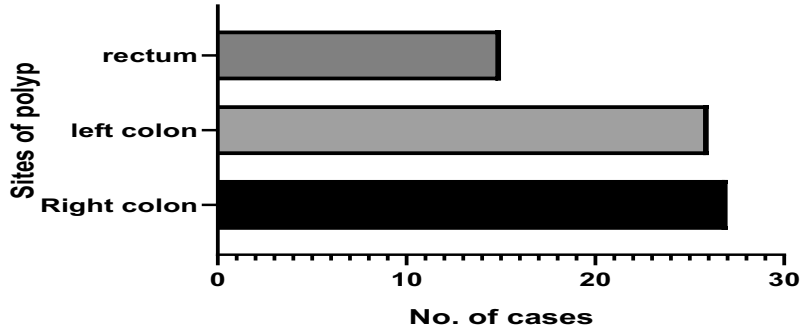

Figure 2. Total number of cases diagnosed with polyp.

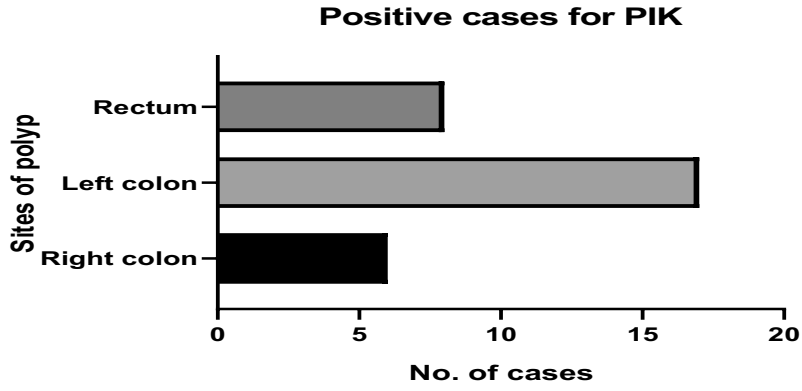

Figure 3. The number of positive cases for PIK according to the site of the polyp.

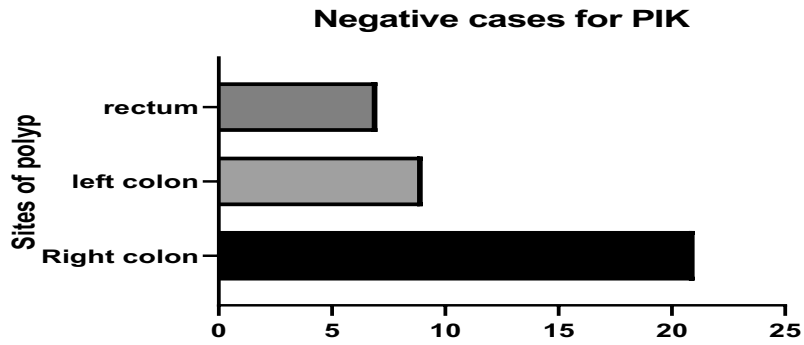

Figure 4. The number of negative cases for PIK staining according to the site of the polyp.

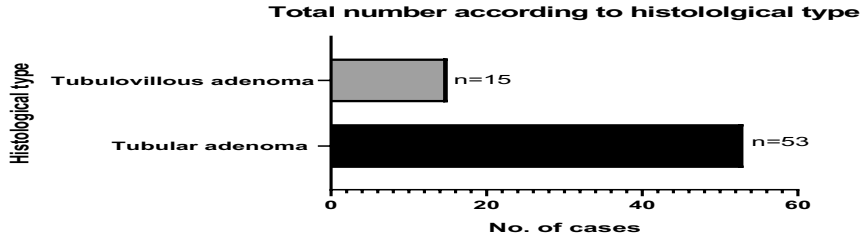

Figure 5. Number of cases according to histological types.

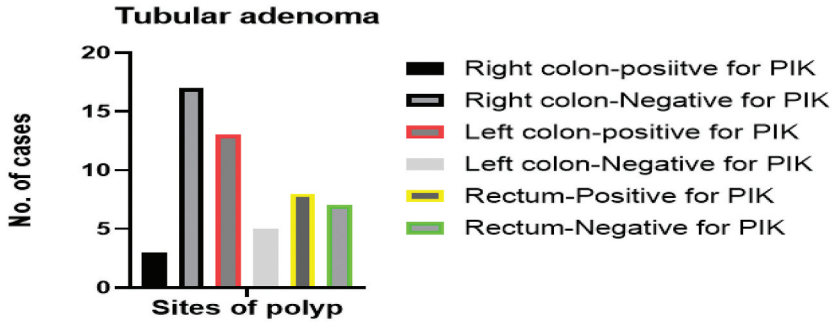

Figure 6. The number of positive and negative cases for PIK staining according to histological type-Tubular adenoma.

the negative ones. All cases in the rectum were diagnosed as tubular adenoma, and the number of cases given positive was roughly equal to those given negative. Concerning tubulovillous, Figure 7 shows no significant differences between positive and negative cases in both the left and right colon. No cases in the rectum were diagnosed as tubulovillous adenoma, Figures 7–11.

Correlations between PIK staining intensity, age, and sex

A substantial inverse correlation was observed between the ages of patients and their gender, indicating an increased prevalence of polyp cases in females compared to males with advancing age. Conversely, a significant direct correlation was discerned between patient age

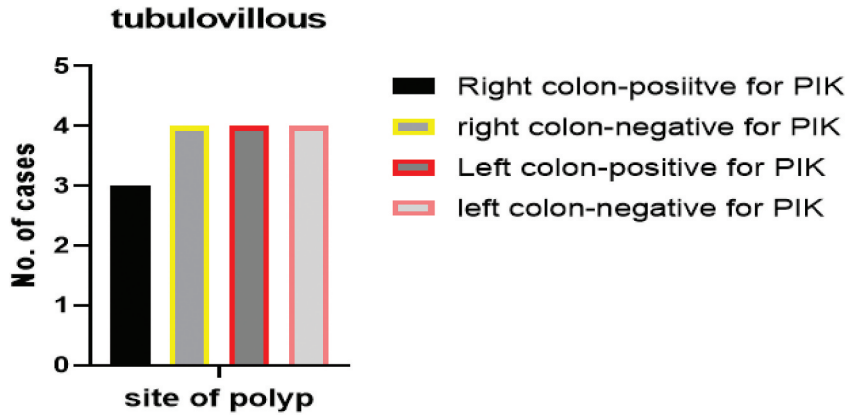

Figure 7. The number of positive and negative cases for PIK staining according to histological type tubulovillous.

Table 2. Correlations between PIK staining intensity, age, and sex.

		Age	Sex	Intensity	Results
Age	r^2		−0.258*	0.926**	0.873**
	p		0.046	.000	0.000
	N		68	68	68
Sex	r^2	−0.258*		−0.106	−0.240
	p	0.046		0.422	0.065
	N	68		68	68
Intensity	r^2	0.926**	−0.106		0.943**
	p	0.000	0.422		0.000
	N	68	68		68

* Correlation is significant at the 0.05 level.
** Correlation is significant at the 0.01 level.

and the intensity of PIK staining, as well as the overall results (positive PIK staining) (*P* value < 0.01). Table 2 shows the data about the correlation between gender, staining intensity, and the staining results. Despite observing a non-significant inverse correlation between the intensity of PIK staining and the results of PIK staining, there was a substantial direct correlation between the intensity of PIK staining and the staining results (p-value <0.01). High intensity of PIK staining generally yielded a positive result for PIK staining. This is contingent upon specific considerations in the materials and methods section, which dictate whether the PIK staining results are positive or negative.

Discussion

A polyp is a mass that protrudes into the lumen of a hollow duct or organ. Colorectal polyps are classified according to histological properties as neoplastic (malignant potential) or non-neo-plastic, including hyperplastic, inflammatory, or hamartomatous polyps. As with any disease in the human body, when diagnosed at the early stages of development, the treatment protocol becomes easy and simple also, with low side effects when compared with the final stages of diseases development, therefore it so important to diagnose the neoplastic polyps in the early stage of development [18]. Thus, the current study attempted to show the role of *PIK3CA*

expression in colorectal polyp development. The patient group was divided into three age groups (50–59 years), (60–69 years), and (70–80 years), including 35 patients, 7 patients, and 24 patients, respectively. In general, age classification in the current study agrees with a study that found a high prevalence of colorectal polyp cases diagnosed in the age above 50 years. The current study is compatible with the results of recent studies [19,20]. The variations of cases in each age group may occur due to many causes; colorectal polyps diagnosed accidentally through colonoscopy screening make the diagnosis don't have a specific standard. A possible second cause may be the small sample size, which can reflect a nonspecific and real representation of the distribution according to age group.

Colorectal polyp, according to the site

Our results show the presence of polyps in the colon is as follows: the right colon had 27 cases, and the left colon 26 cases, while in the rectum there were only 15 cases, as shown in Figure 2. The possible explanation for these results may belong to anatomical causes because the proximally right colon from the small intestine makes the right colon the first site exposed to intestinal content (digestive enzymes, other chemical substances), which came from the small intestine. GIT content may cause damage to epithelial cells. Replacement of damaged cells as a normal physiological response with other new cells, with time, can lead to newly generated cells having abnormal gene(s), possibly resulting in polyp overgrowth. The present results are consistent with a study by [21]and opposite to the study reported by Lorentzen and his colleagues, who found that the number of cases diagnosed in the proximal colon was 21 (10.3%) cases, while 181 (89.7%) cases were diagnosed in the distal colon [22].

Positive and negative PIK staining according to the site of the polyp

The gene *PIK3CA*, encoding the alpha catalytic subunit of phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K), plays an important role in developing many malignancies. The *PIK3CA* gene is found at locus 3q26.32 on the long arm of chromosome number 3. *PIK3CA* mutations have been found in many malignancies, including the most common cancers, such as breast, endometrial, and colorectal cancers [23]. The PIK staining in the current study shows that all control group cases were negative, while in the patient's group, all cases were positive, as

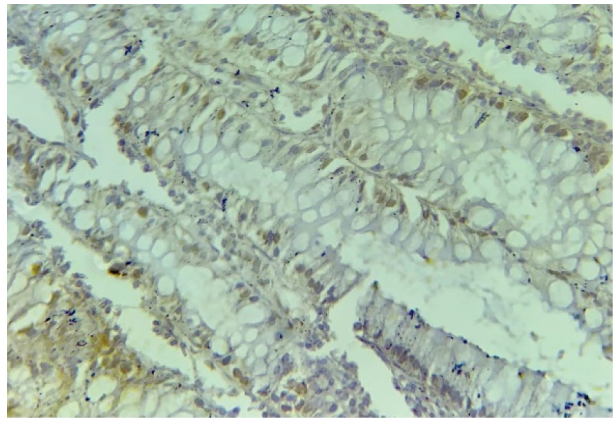

Figure 8. Adenomatous polyp positive for *PIK3CA*, compound microscope (10x).

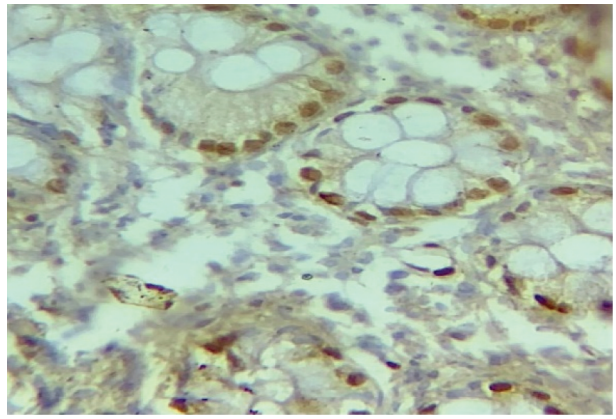

Figure 9. Adenomatous polyp positive for *PIK3CA*, compound microscope (40x).

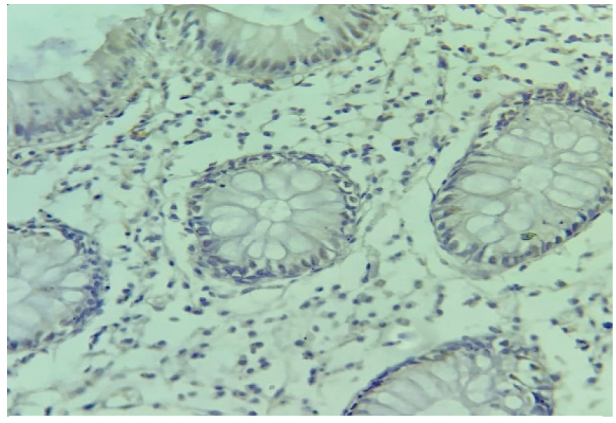

Figure 10. Adenomatous polyp negative for *PIK3CA*, compound microscope (40x).

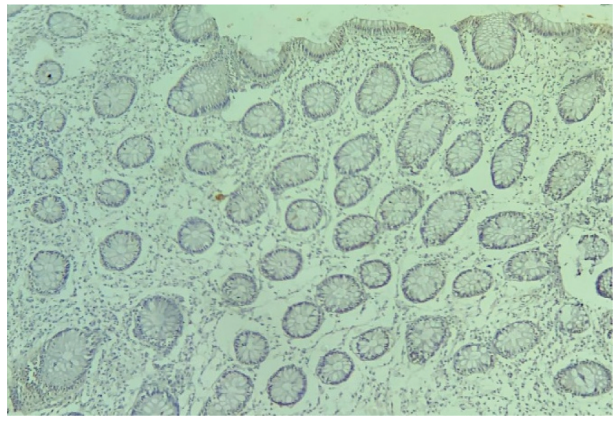

Figure 11. Colitis negative for *PIK3CA*, compound microscope(10x).

shown in Figures (8–11). These results may indicate a possible role of the *PIK3CA* gene in tumor development, and these results are consistent with a study achieved by [24].

The number of cases positive for PIK staining in the left colon is higher than in the rectum, and the cases positive in the rectum are higher than in the right colon, as shown in Figure 3. The probable explanation for these results is the limited overgrowth caused by a genetic abnormality (mutations) in genes other than PIK, such as Kras, TP53, and so on [25,26].

When comparing site and positive and negative PIK staining, we found that the number of negative cases was higher than those shown positive in the right colon. In comparison, in the left colon, the number of positive cases was higher than the number of negative cases. Finally, the number of positive cases equals negative cases, as shown in Figure 4.

Distribution of cases according to histological types

In the current study, the majority histological type was tubular adenoma ($n = 53$), and only 15 cases were tubulovillous adenoma, as shown in Figure 5. A study achieved by [22]Found 121 cases of tubular adenoma, while 31 cases have tubulovillous adenoma. Histological type-tubular adenoma and results of PIK staining among the site of the polyp are shown in Figure 6. The number of cases with tubular adenoma negative for PIK staining was significantly higher than those positive one in the right colon. The cases with positive staining were significantly higher in the left colon than those with negative. Finally, all cases in the rectum were diagnosed as tubular adenoma, and the number of positive cases was roughly equal to that of negative cases ($p < 0.05$). In the tubulovillous histological type, the number of positive cases in the right colon is lower than negative cases. In contrast, the positive cases are higher than the negative cases in the left colon. There were no significant differences between cases that were diagnosed positive and negative in the right and left colon ($p < 0.05$). No cases in the rectum were diagnosed as tubulovillous adenoma. Based on our knowledge, the current study was the first to investigate *PIK3CA* expression among histological types of colorectal polyps.

Correlations between PIK staining intensity, age, and sex

The current results show a significant negative correlation between the ages of patients and sex; this means the number of polyp cases increased with age in females more than in males. We found that as people age, the intensity of PIK staining increases, and the likelihood of a positive PIK staining result also increases. However, there was no significant correlation between sex and PIK staining intensity or results. We also found that a higher intensity of PIK staining is significantly correlated with a positive PIK staining result. PIK staining results depend on certain factors, as described in the materials and methods section.

Conclusion

There is a significant difference in cases that gave positive for PIK staining between patients and the control group. The positive cases of PIK staining in the patient's group were higher than in the control group; therefore, the current study suggests that the *PIK3CA* gene has a role in forming polyps (benign tumors) that may give rise to possible malignant tumor development.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This research received no external funding.

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Ethical approval

Ethical approval (30/2022) was provided by the Faculty of Medical Technology, The Islamic University of Najaf, Iraq. We obtained written informed consent from all participants prior enrolled in the current study.

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