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Research Article

The Sensitivity of Immunohistochemical Expression of *p53* as an Indicator of the Malignant Potential of Gastric Hyperplastic Polyps: A Retrospective Study

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Abstract

Background: Hyperplastic polyps account for 30–93% of gastric polyps. Recently, there have been studies about the development of dysplasia in this type of polyp. Every cell in the body contains the p53 gene, which has anti-cancer properties. ***Objective***: The sensitivity of immunohistochemical expression of p53 is evaluated among gastric hyperplastic polyps with and without dysplasia and gastric adenomatous polyps to know its usefulness as a diagnostic marker. ***Methods***: A retrospective cross-sectional study was done on fifty formalin-fixed paraffin-embedded blocks of gastric polyps (44 hyperplastic polyps without dysplasia, 3 hyperplastic polyps with dysplasia, and 3 adenomas). Cases were collected from the archives of the pathology department from June 2019 to July 2023. Additional sections of the block were immunostained with P53 protein. ***Results***: Fifty paraffin blocks from patients with gastric polyps (17 males, 33 females) were included in the study. 44 of the cases are classified as hyperplastic polyps, 3 as hyperplastic polyps with dysplasia, and 3 as adenomatous polyps. Most gastric hyperplastic polyps showed staining in different scores. All gastric hyperplastic polyps with dysplasia showed nuclear staining, whereas two out of three gastric adenomatous polyps showed no staining. ***Conclusion***: There was no significant association between p53 protein expression and the type of gastric polyps, the presence of intestinal metaplasia, or *H. pylori*. However, it has a significant correlation with the size of the polyps.

Keywords: Dysplasia, Gastric polyps, Hyperplastic polyps, Immunohistochemistry, p53.

حساسية التعبير المناعي الكيميائي ل p53 كمؤشر على الإمكانات الخبيثة للأورام الحميدة المفرطة التنسج في المعدة: دراسة بأثر رجعي

الخلاصة

الخلفية: الاورام الحميدة المفرطة التنسج تمثل 30-93 ٪ من الاورام الحميدة في المعدة. في الآونة الأخيرة، كانت هناك دراسات حول تطور خلل التنسج في هذا النوع من الاورام الحميدة. تحتوي كل خلية في الجسم على جين p53 ، الذي له خصائص مضادة للسرطان. **الهدف**: يتم تقييم حساسية التعبير الكيميائي المناعي ل p53 بين الاورام الحميدة المفرطة التنسج في المعدة مع وبدون خلل التنسج والأورام الحميدة الغدية في المعدة لمعرفة فائدتها كعلامة تشخيصية. **الطريقة**: أجريت دراسة مقطعية بأثر رجعي على خمسين كتلة مدمجة بالبارافين مثبتة بالفورمالين من سلائل المعدة (44 سلائل مفرطة التنسج بدون خلل التنسج ، و 3 سلائل مفرطة التنسج مع خلل التنسج ، و 3 أورام غدية). تم جمع الحالات من أرشيف قسم علم الأمراض من يونيو 2019 إلى يوليو 2023. تم تلطيخ أقسام إضافية من الكتلة ببروتين p53. **النتائج**: تم تضمين خمسين كتلة من البارافين من المرضى الذين يعانون من سلائل المعدة (17 من الذكور و 33 من الإناث) في الدراسة. تصنف 44 من الحالات على أنها سلائل مفرطة التنسج ، و 3 سلائل مفرطة التنسج مع خلل التنسج، و 3 سلائل غدية. أظهرت معظم الاورام الحميدة المفرطة في المعدة تلطيخ في درجات مختلفة. أظهرت جميع الاورام الحميدة المفرطة التنسج في المعدة مع خلل التنسج تلطيخا نوويا ، في حين أن اثنين من كل ثلاثة سلائل غدية في المعدة لم يظهروا أي تلطيخ. **الأستنتاج**: لم يكن هناك ارتباط كبير بين تعبير بروتين p53 ونوع الاورام الحميدة في المعدة ، أو وجود حؤول الأمعاء ، أو *H. pylori*. ومع ذلك ، فإنه له علاقة كبيرة مع حجم الاورام الحميدة.

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INTRODUCTION

Among benign gastric epithelial polyps, hyperplastic polyps represent 30–93% [1]. Helicobacter pylori gastritis, autoimmune metaplastic atrophic gastritis, Ménétrier disease, and gastric surgery are the most important risk factors for gastric hyperplastic polyps [2]. Usually, endoscopy accidentally detects these polyps, though anemia may be a presenting feature [1]. From a histological perspective, gastric hyperplastic polyps consist of polypoid lesions, characterized by dilated glands with architectural distortion and a foveolar epithelial lining with minimal nuclear atypia. Intestinal metaplasia was reported in 16% of the cases [3]. Gastric hyperplastic polyps are not entirely innocent, as they may locally recur and contain dysplasia or carcinoma foci. Polyps measuring more than 1 cm are associated with more than 50% risk of local recurrence, 1.5-4.4 % prevalence of dysplasia (both low and high grades),

and between 1.1-2.1% prevalence of cancer [2]. However, the pathogenesis of the neoplastic change is not well understood [1]. p53 is a transcription factor that has a vital role in the protection against the development of malignancy. When the cell is injured and, as a result, DNA is damaged, p53 is activated and causes either arrest of the cell cycle to allow the cell to repair its DNA to prevent the transfer of this damage (mutation) to its daughter cells or the cell will undergo apoptosis if the DNA damage is beyond repair [4-8]. The p53 gene produces a p53 protein with a half-life of 20 minutes; thus, in our study, we evaluate the immunohistochemical expression of p53 in gastric hyperplastic polyps, both with and without consequence, and in gastric adenomatous polyps to ascertain the effectiveness of p53 as a diagnostic marker. A mutated p53 gene may produce an aberrant protein that has a longer half-life, is aggregated in the cell and, as a consequence, is easily discovered by immunohistochemistry [9]. In this study, the immunohistochemical expression of p53 is assessed among gastric hyperplastic polyps with and without dysplasia and gastric adenomatous polyps to determine the utility of p53 as a diagnostic marker.

METHODS

Study design and setting

This study is a retrospective, cross-sectional study that involves collecting the pathological reports and paraffin-embedded blocks of 50 gastric polyps archived in the pathology department. These polyps were diagnosed by esophagogastroduodenoscopy and biopsy from June 2019 to July 2023. The polyps were from 17 males and 33 females, aged between 17 and 74 years. The study includes forty-four hyperplastic polyps without dysplasia, three hyperplastic polyps with dysplasia, and three adenomatous polyps. The diagnoses of these polyps were done by expert pathologists in the hospital.

Ethical consideration

The ethical committee of the scientific unit and medical ethics of Al-Kindy College of Medicine has approved this retrospective study (approval no. 185, dated 1st September 2021). The study does not involve any harm or risk to any patient, nor does it disclose any information about them.

Sample collection and outcomes measurement

In this study, we assess the immunohistochemical expression of p53 in gastric hyperplastic polyps with and without dysplasia, as well as gastric adenomatous polyps, to determine the utility of P53 as a diagnostic mark. The paraffin blocks were cut in 4-micron sections and stained with p53 immunohistochemically using an antibody P53 (Clone: DO-7 (1)). Isotype: IgG2b, kappa. The staining steps and incubation times are pre-programmed into the Autostainer Link software. The recommended reagent application volume is 1 x 200µL or 2 x 150µL per slide. Counterstaining in hematoxylin was done using EnVision FLEX Hematoxylin (Link) (Code K8008). Positive and negative control tissues as well as negative control reagents are run simultaneously using the same protocol as the patient specimens. The positive control tissues include colon and colon adenocarcinoma. The recommended negative control reagent is FLEX Negative Control, Mouse (Link) (Code IR750). (https://www.agilent.com/store/en_US/Prod-IR61661-2/IR61661-2).

Interpretation of Immunohistochemical staining

Two pathologists examine the immunohistochemistry slides for P53 protein and score the nuclear staining of p53 protein in the hot spot using the following criteria: 0: when there is no nuclear or cytoplasmic staining on the cells; 1+: when less than or equal to 30% of the cells are stained; 2+: when 31-70% of the cells are stained; 3+: when more than 70% of the cells are stained [10]. To be more statistically significant, p53 was considered to be absent if it scores 0 and present if any nuclear staining appears.

Statistical analysis

Data was introduced into Microsoft Excel sheet 2019 and loaded into SPSS (Statistical Package for Social Sciences) version 24. Parametric data was presented as mean and standard deviation. Categorical data was presented as numbers and percentages. The chi-square test and Fisher exact test were used to analyze qualitative data, and a $p<0.05$ was considered as discrimination of significance. We used Spearman correlation to correlate non-parametric variables. Pearson correlation was used to correlate parametric variables, which was considered significant at $p<0.05$.

RESULTS

The study includes 50 patients (17 males and 33 females) who underwent upper endoscopy for their symptoms and during the procedure, a gastric polyp was discovered, removed, and sent for histopathology diagnosis. The patients' age ranges from 17 to 74 years. The histopathological diagnosis of the polyps using routine H&E slides was the following: 44 hyperplastic polyps (88% of the cases), 3 hyperplastic polyps with dysplasia (6%), and 3 adenomatous polyps (6%) (Figure 1).

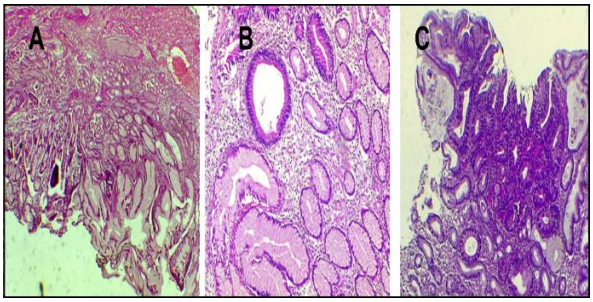

Figure 1: Types of gastric polyps (H&E, X4 - X10 HPF). A) Hyperplastic polyp; B) Hyperplastic polyp with dysplasia; C) Gastric adenoma.

The size of these polyps, as mentioned in the pathological reports, was from 2 to 20 mm. *Helicobacter pylori* was present in 21 cases (42%), with intestinal metaplasia being reported in 18 cases (36%) (Table 1). There was no significant association between the type of the polyp and the gender, age of the patients, or size of the polyp. The same pertains to the presence of *H. pylori* and intestinal metaplasia (Table 2). Regarding nuclear staining of p53 protein immunohistochemically, most gastric hyperplastic polyps (32 out of 44 polyps) show staining in different scores (Figure 2).

All three gastric hyperplastic polyps with dysplasia show nuclear staining (score range 2+-3+), while two out of three gastric adenomatous polyps show absent staining (Figure 1). No significant association was observed between the p53 immunostaining with the type of the polyp and the presence of *H. pylori* or intestinal metaplasia (Figure 3).

Table 1: Baseline demographic, clinical, and pathologic characteristics of the study cases

Characters		*n*(%) Median (Range)
Age group	≤ 45 years	24(48)
	> 45 years	26(52)
	Median Age in years	46(17-74)
Gender	Male	17(34)
	Female	33(66)
Polyp type	Hyperplastic	44(88)
	Hyperplastic with dysplasia	3(6)
	Adenomatous	3(6)
Polyp size	≤ 6 mm	25(50)
	> 6 mm	25(50)
Median polyp size in mm		5.5(2.0-20.0)
H. Pylori	Absent	29(58)
	Present	21(42)
Intestinal metaplasia	Absent	32(64)
	Present	18(36)
p53	Absent	14(28)
	Present	36(72)
p53 Score	0	14(28)
	+1	14(28)
	+2	10(20)
	+3	12(24)

Table 2: Factors associated with the type of the polyp

Variable		Polyp type			*p*-value
		Hyperplastic	Hyperplastic with dysplasia	Adenomatous	
Age group	≤ 45 years	22(50.0)	1(33.3)	1(33.3)	1
	> 45 years	22(50.0)	2(66.7)	2(66.7)	
Gender	Male	15(34.1)	0 (0.0)	2(66.7)	0.326
	Female	29(65.9)	3(100)	1(33.3)	
Polyp size	≤ 6 mm	23(52.3)	0(0.0)	2(66.7)	0.414
	> 6 mm	21(47.7)	3(100)	1(33.3)	
H. Pylori	Absent	25(56.8)	2(66.7)	2(66.7)	1
	Present	19(43.2)	1(33.3)	1(33.3)	
Intestinal metaplasia	Absent	29(65.9)	2(66.7)	1(33.3)	0.792
	Present	15(34.1)	1(33.3)	2(66.7)	
P53	Absent	12(27.3)	0(0.0)	2(66.7)	0.196
	Present	32(72.7)	3(100)	1(33.3)	

Values were expressed as frequencies and percentages.

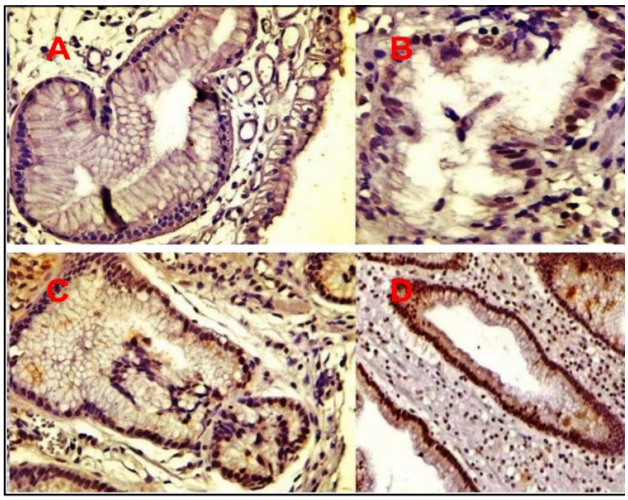

Figure 2: immunohistochemical expression of P53 protein (X 40 HPF). A) Score 0; B) Score 1+; C) Score 2+; D) Score 3+.

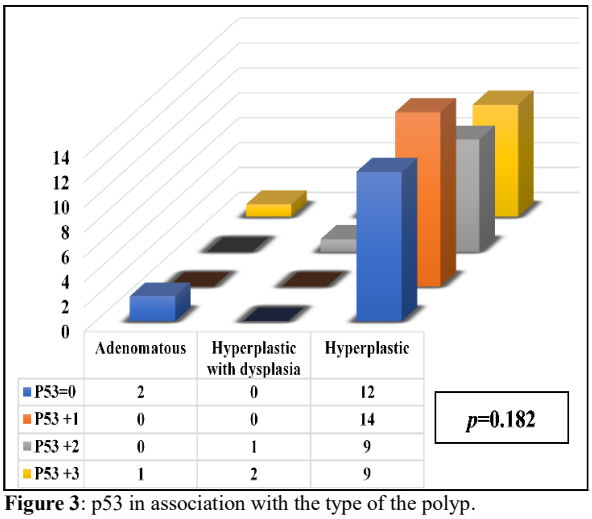

Figure 3: p53 in association with the type of the polyp.

However, there was a significant association with the size of the polyp (Tables 3 and 4).

DISCUSSION

Gastric hyperplastic polyps represent the most common type of gastric polyps (up to 75%). *H. pylori* infection is closely related to its etiology, similar to gastric cancer [11]. Although few case reports cite malignant transformation of gastric hyperplastic polyps [12], these are still considered benign lesions unless dysplasia is present. Other risk factors for gastric cancer, such as smoking and alcohol, dietary factors, atrophic gastritis, partial gastrectomy, Epstein-Barr virus, or genetic predisposition, should be searched for in the clinical assessment of the patient [13].

Table 3: The correlations between P53 with the intestinal metaplasia, polyp type, and polyp size

Spearman's rho	Intestinal metaplasia	r_s *p*-value	0.057 0.695
Spearman's rho	Polyp type	r_s *p*-value	0.111 0.443
Pearson coefficient	Polyp size	*r* *p*-value	0.591 <0.001

Table 4: P 53 in association with the polyp size, type of the polyp, intestinal metaplasia, and *H. Pylori*

Variables		p53				*p*-value
		0	+1	+2	+3	
Polyp size	≤ 6 mm	10(71.4)	9(64.3)	4(40)	2(16.7)	0.024
	> 6 mm	4(28.6)	5(35.7)	6(60)	10(83.3)	
Polyp type	Hyperplastic	12(85.7)	14(100)	9(90)	9(75)	0.182
	Hyperplastic with dysplasia	0(0.0)	0(0.0)	1(10)	2(16.7)	
	Adenomatous	2(14.3)	0(0.0)	0(0.0)	1(8.3)	
Intestinal metaplasia	Absent	8(57.1)	12(85.7)	5(50)	7(58.3)	0.233
	Present	6(42.9)	2(14.3)	5(50)	5(41.7)	
H. Pylori	Absent	8(57.1)	8(57.1)	5 (50)	8(66.7)	0.873
	Present	6(42.9)	6(42.9)	5 (50)	4(33.3)	

Values were expressed as frequencies and percentages.

In our study, approximately two-thirds of the patients with gastric polyps were female (33 out of 50 patients). This result is in agreement with a study done by Hu *et al.*, which showed a female-to-male ratio of 1.5/1.0 [1]. The median age in our study is 46 years (range 17-74), while in a study by Joao *et al*., the median age was 67 years (range 35-87). In our study, there was no significant association between the type of the polyp and the size of the polyp, as well as the presence of *H. pylori* or intestinal metaplasia. These results reiterate those reported by Ahn *et al*., which found no association between the presence of dysplasia/neoplastic change with the occurrence of *H. pylori* and intestinal metaplasia in the background gastric mucosa [14]. Hu *et al*. also reported no association between age, gender, *H. pylori* infection, and the polyps that show neoplastic transformation [1]. In a study performed by Joao *et al*., the results revealed large polyp size (more than 25 mm) and intestinal metaplasia is associated with neoplastic transformation of hyperplastic polyps. This difference between his study and us could be explained by none of our studied polyps being larger than 25 mm (the largest was 20 mm); and regarding intestinal metaplasia, a Japanese study mentioned a second pathway of neoplastic transformation in gastric hyperplastic polyps in which the neoplastic change of these polyps develops through a hyperplasia-dysplasia-carcinoma sequence rather than the usual pathway (intestinal metaplasia-dysplasia-carcinoma) [15]. Our findings indicate that most gastric hyperplastic polyps (32 out of 44 polyps) show nuclear staining of p53. All hyperplastic polyps with dysplasia show nuclear staining ranging from 2+ to 3+ (Figure 4), while two out of three adenomatous polyps show absent nuclear staining. The previous studies concluded increased expression of p53 from hyperplastic polyps to hyperplastic polyps with dysplasia, specifically in the dysplastic areas [16–18]. In contrast, none of the gastric polyps in the Saab et al. study, whether hyperplastic polyps without dysplasia or with dysplasia, show nuclear staining of p53 in more than 10% of the epithelium. This difference might relate to the difference in the interpretation of the score of p53 immunohistochemically. In this study, there was no significant association between the p53 protein expression with the type of the polyp and the presence of *H. pylori* or intestinal metaplasia. No previous studies correlate the expression of p53 with these parameters, as far as we know. However, there was a significant association with the size of the polyp; this result confirms the data reported in a study by Murakami *et al*. that reported a positive correlation between the size of 50 hyperplastic polyps without dysplasia and p53 expression.

Study limitations

Many factors contributed to the small number of cases, including the use of a single tertiary hospital, the absence of many cases from slide reviews conducted outside the hospital, and the lack of a screening program for gastric polyps and cancer in Iraq. The study lacks uniformity in interpreting p53 scores through immunohistochemistry, and there is a dearth of published research on this crucial subject.

Conclusion

There was no significant association between p53 protein immunoexpression and the type of gastric polyps, intestinal metaplasia, or *H. pylori*. However, it has a significant association with the size of the polyps. Other studies must validate our findings to establish the effectiveness of p53 as a diagnostic marker for identifying dysplasia in unclear cases. Additionally, we recommend studying other potential markers immunohistochemically and molecularly to investigate their diagnostic or prognostic value.

Conflict of interests

No conflict of interests was declared by the authors.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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