



An Immunohistochemical Assessment of Ki-67 and P53 Over-expression in *Helicobacter pylori*-positive Gastritis

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Abstract

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AIM: *Helicobacter pylori* infection of the stomach is a common disease and the resulting changes from it are many and deserve to be in the focus of researchers' attention, to assess the expression of mutant P53 protein and Ki-67 markers in patients with gastritis secondary to *H. pylori*.

METHODS: Thirty samples with positive *H. pylori* gastritis were included in a retrospective study in Mosul/Iraq. The histological parameters were assessed using the Sydney system, then, the expression of Ki-67 and P53 expression was studied by immunohistochemical methods. The significance level was appointed at (0.05).

RESULTS: Ki-67 and P53 expression were found in 83.3% of the total cases. The study results show that 92% of positive Ki-67 and P53 cases had chronic inflammatory cell infiltration, polymorph nuclear cells infiltration, and atrophy. Whereas 96% of positive Ki-67 cases had no metaplasia, 92% of the positive P53 cases had no metaplasia. The results also showed that only 16% of the positive Ki-67 cases had dysplastic changes, and 24% of the positive cases of P53 cases were showed dysplasia. whenever P53 was negative; there is neither metaplasia nor dysplasia in the tissue, this does not apply to Ki-67 negative cases.

CONCLUSIONS: Ki-67 and P53 expressions increase with chronicity of *Helicobacter pylori*-positive gastritis. P53 expression is amplified when atrophy is present in these samples.

Introduction

Gastritis is defined as inflammation of the stomach mucosa, it could be acute or chronic [1]. Chronic gastritis is still common in developing countries [1]. Gastritis is believed to effect 50% of people in the world and the prevalence increases with age [2]. Gastritis might be complicated by bleeding, ulcer, and stomach cancer, so it is worth studying and exploring its causes to pave the way for treatment [1].

Although *Helicobacter pylori* (HP) is one of the most common chronic infections worldwide (about 50% of the population is infected by HP) and is the most common cause for gastritis [3]; however, almost two-thirds of patients with negative HP were found to have gastritis. Other causes of gastritis include ingestion of non-steroidal anti-inflammatory drugs, smoking, narcotics, alcohol, and autoimmune diseases [4], [5], [6], [7], [8].

Chronic gastritis is associated with neutrophil infiltration; with different effects according to HP strain, the aggressive strain will cause atrophic gastritis, in this situation, the lost glands replaced by new immature glandular and epithelial cells look such as glands of intestinal tissues and called intestinal metaplasia which

is found in end-stage disease and are considered as premalignant lesions [9], [10].

The long-term presence of HP leads to persistent damage and hyperproliferation of the lining epithelium of the stomach, which will lead to an increase in the occurrence of mutations and the development of gastric cancer [11]. P53 is an important prognostic factor in many tumors, one of them is early gastric adenocarcinoma [12], [13]. The p53 gene activation occurs after damaging the DNA, which, in turn, leads to cell cycle arrest in phase G1 and repairs the damaged DNA or cell apoptosis. Recently, studies have shown that HP infection plays a direct role in p53 mutagenesis in patients with intestinal metaplasia and dysplasia [14], [15].

Ki-67 protein expression is linked to cell proliferation, and while it is missing in resting cells, it is present throughout the cell cycle's active stages, making it a perfect biomarker for cell proliferation [16]. Studies showed that expression of Ki-67 is higher in intestinal metaplasia in the specimens with positive HP infection in comparison to negative HP samples, this illustrates the effect of HP infection on the expression of Ki-67 during the precancerous phases [17], [18], [19], [20]. The previous findings have shown that the expressions of P53 and Ki-67 were associated with each other. However, these markers alone had no diagnostic value [21], [22].

Aim

This study aims to assess the mucosal expression of mutant p53 protein and Ki-67 markers in patients with gastritis secondary to HP infection, the results were correlated with the age and gender of the patients and with some histological properties guided by the Sydney system.

Materials and Methods

Study setting and design

At first, all necessary official approvals were obtained before conducting the Mosul research from the College of Medicine of Mosul. Tiny pieces of samples suspected of gastritis were obtained by endoscope in this retrospective study. The cases were collected from Nineveh governorate private laboratories and teaching hospitals, then processed in all procedures needed together with data analysis from October 2020 to April 2021. Clinical data were collected including age and sex.

Histopathology and immune staining

At first, H&E staining was performed for each sample, if the diagnosis of gastritis was established, then searching for availability of HP with Giemsa stain, positive cases of HP gastritis only included in this study. The histological parameters were evaluated using the Sydney system: Chronic inflammation, PNM (polymorph nuclear cells), atrophy, and metaplasia, in which the cases were divided into four grades as follows: 0: not present, 1: mild, 2: moderate, and 3: severe. Dysplasia assessment was added to the Sydney system.

After collecting 30 cases of HP positive gastritis, the Ki-67 and P53 over-expressions were studied by an immunohistochemical method using Dako monoclonal antibody for Ki-67 and P53 Protein.

Table 1: Age distribution with Ki-67 expression

Age groups (years)	n	Ki-67+ve, n (%)	Ki-67-ve, n (%)	p
<28	4	4 (16.0)	0	0.039
28–41	6	5 (20.0)	1 (20.0)	
42–55	11	9 (36.0)	2 (40.0)	
56–69	5	4 (16.0)	1 (20.0)	
≥70	4	3 (12.0)	1 (20.0)	
Total	30	25 (100.0)	5 (100.0)	

Immune-staining assessment

Ki-67 and P53 staining intensity was graded as follows (independently): 0 for negative staining, 1 for mild staining, 2 for moderate staining, and 3 for high staining. [23]. The staining distribution was categorized

as follows: 0, < 5%; 1, 5%–25%; 2, 25%–50%; 3, 50%–75%; and 4, > 75%, and the final score was obtained by multiplying the intensity score by the distribution score. The final results were within the range of 0–12, scores greater than 4 were considered as a positive expression for that marker, and <4 were considered negative expressions for that marker [24].

Statistical analysis

Statistical analysis was done with Chi-square test and (F) test as required, using Minitab 18th edition, with $p < 0.05$ used as a significant association in the results.

Results

Marker expression

In this study, 30 patients with HP-positive gastritis were recovered, all samples were antral biopsies, Ki-67 expression was found in 83.3% of the total cases, the same for the P53 marker, in which also 83.3% of the cases were positive. The detection of Ki-67 concordance with P53 was statistically in fair agreement ($\kappa=0.267$) (Figure 1).

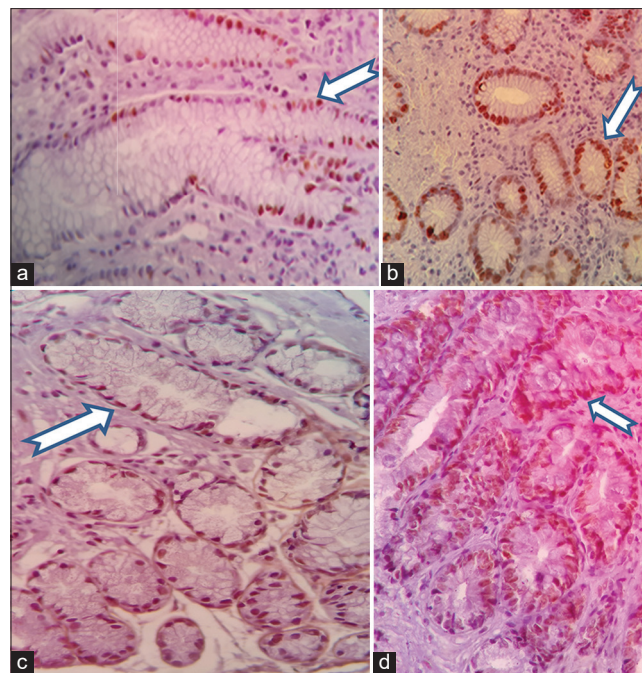


Figure 1: *H. pylori* positive gastritis with weak Ki-67 expression magnification-400. shows four cases with weak and strong expression of Ki-67 and P53 expression in this figure (a), strong Ki-67 expression (b), weak P53 expression (c), and strong P53 expression (d) appeared as brown color (arrows) magnification-400

Patient's age and sex

The age of the patients ranged from 14 to 84 years with a mean of 46 years ± 17.2 (Tables 1 and 2), the largest group of Ki-67 positive cases (36%) was in the age group (42–55) years, again the largest group of P53 positive cases (40%) also was in the age group (42–55) years, with significant p-values (0.039) and (0.019), respectively.

Table 2: Age distribution with P53 expression

Age groups (years)	n	P53+ve, n (%)	P53-ve, n (%)	p
<28	4	4 (16.0)	0	0.019
28–41	6	4 (16.0)	2 (40.0)	
42–55	11	10 (40.0)	1 (20.0)	
56–69	5	4 (16.0)	1 (20.0)	
≥70	4	3 (12.0)	1 (20.0)	
Total	30	25 (100.0)	5 (100.0)	

About 53.3% of the cases were female with 56% of the positivity in both markers, while 60 % of negative cases in both markers were male, with no significant p-value (0.409) (Figure 2).

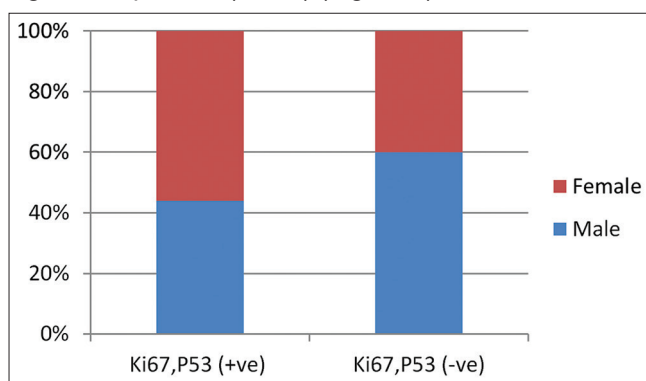


Figure 2: Sex distribution with Ki-67 and P53 expression, p-value (0.409)

Histological examination

About 2/3 of cases with positive Ki-67 expression had grade 2 or 3 of chronic inflammatory cells, PMN cells infiltrations, atrophy with p-values (0.014) that were significant, (0.227) not significant, and (0.131) not significant, respectively. While 96% of them

Table 3: Sydney system with Ki-67 expression

Sydney system	n	Ki-67+ve, n (%)	Ki-67-ve, n (%)	p
Chronic inflammation				0.014
0	1	0	1 (20.0)	
1	5	3 (12.0)	2 (40.0)	
2	9	9 (36.0)	0	
3	15	13 (52.0)	2 (40.0)	
Total	30	25 (100.0)	5 (100.0)	
P.M.N.				0.227
0	5	2 (8.0)	3 (60.0)	
1	7	7 (28.0)	0	
2	12	10 (40.0)	2 (40.0)	
3	6	6 (24.0)	0	
Total	30	25 (100.0)	5 (100.0)	
Atrophy				0.131
0	4	1 (4.0)	3 (60.0)	
1	7	7 (28.0)	0	
2	13	11 (44.0)	2 (40.0)	
3	6	6 (24.0)	0	
Total	30	25 (100.0)	5 (100.0)	
Metaplasia				0.013
0	28	24 (96.0)	4 (80.0)	
1	1	0 (0.0)	1 (20.0)	
2	1	1 (4.0)	0	
3	0	0	0	
Total	30	25 (100.0)	5 (100.0)	

had no metaplasia with significant p-value (0.013). On the other hand, more than half of the negative Ki-67 cases had no infiltration of PMN cells, no atrophy, no metaplasia, and only 20% of them had no infiltration of chronic inflammatory cells (Table 3).

The study results show that 92% of positive P53 cases had chronic inflammatory cells, PMN cells infiltration, and atrophy with p-values (0.020) significant, (0.110) non-significant, and (0.078) nonsignificant, respectively. The opposite was true for metaplasia, in which 92% of positive cases of P53 cases had no metaplasia with a significant p-value (0.034) (Table 4).

Table 4: Sydney system with P53 expression

Sydney system	n	P53+ve, n (%)	P53-ve, n (%)	p
Chronic inflammation				0.020
0	1	0	1 (20.0)	
1	5	2 (8.0)	3 (60.0)	
2	9	8 (32.0)	1 (20.0)	
3	15	15 (60.0)	0	
Total	30	25 (100.0)	5 (100.0)	
P.M.N.				0.110
0	5	2 (8.0)	3 (60.0)	
1	7	6 (24.0)	1 (20.0)	
2	12	11 (44.0)	1 (20.0)	
3	6	6 (24.0)	0	
Total	30	25 (100.0)	5 (100.0)	
Atrophy				0.078
0	4	1 (4.0)	3 (60.0)	
1	7	5 (20.0)	2 (40.0)	
2	13	13 (52.0)	0	
3	6	6 (24.0)	0	
Total	30	25 (100.0)	5 (100.0)	
Metaplasia				0.034
0	28	23 (92.0)	5 (100.0)	
1	1	1 (4.0)	0	
2	1	1 (4.0)	0	
3	0	0	0	
Total	30	25 (100.0)	5 (100.0)	

Finally, while 16% of positive Ki-67 cases were only showed dysplastic changes, 24 % of positive P53 cases were showed dysplasia, with non-significant p-values (0.074) and (0.467), respectively (Figure 3).

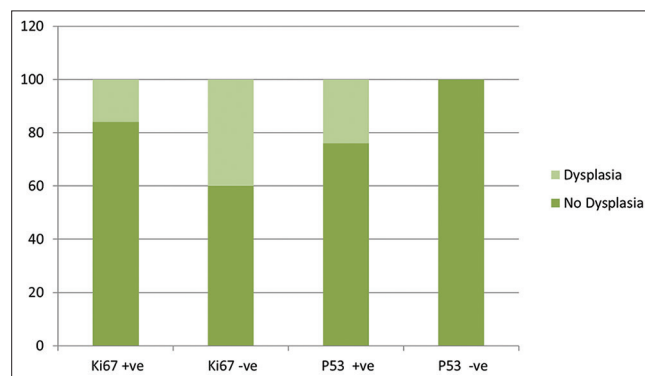


Figure 3: Dysplasia with Ki-67 expression: p-value (0.074), P53 expression: p-value (0.467)

Discussion

Many researchers have studied the expression of Ki-67 and P53 in both positive and negative HP gastritis, gastric ulcer, and cancer. In this study, the expression of Ki-67 and P53 was assessed only in positive HP gastritis and the expression of Ki-67 and P53

was detected in 83.3% of cases, a higher percentage more than many other studies, such as Azza *et al.* [25] study, in which Ki-67 and P53 expression was 54.5% and 72.7%, respectively, Cesar *et al.* [26] study, in which P53 expression was 45%, Afaf *et al.* [27] study, in which P53 expression was 32.7%, while in Morales-Fuentes *et al.* [28] study, the P53 expression was 91% which is more than this study. The differences could be due to the different sample sizes of the cases taken by the studies, different genetic backgrounds, variation in food types between the nations, etc. It is worth saying that despite the similarity of the overall percentage of positive cases for the two markers in the present study, there were positive cases for Ki-67 and negative for P53 and *vice versa* in six cases, and only two cases were negative for both markers.

In this study, the age range from 14 to 84 years with a mean of 46 years \pm 17.2, male-to-female ratio was 7:8, in Morales-Fuentes *et al.* [28] study, the range of age was 55–81 years and male-to-female ratio 5:6, while in Azza *et al.* [25] study, the patients mean age was 53 years \pm 5.1 and male-to-female ratio 1.75:1, and in Zainab *et al.* [29] study, the patients' age range was 11–82 years (mean of 46.5 years) with a male-to-female ratio of 1.38:1. Ki-67 and P53 expressions in this study were significantly detected more in age group 42–55 years, also the expression of both Ki-67 and P53 was seen in females more than males. This discrepancy of results among different studies might be due to bias in data collection of some researches.

In the present study, 86.2% of the studied cases with chronic infiltration of inflammatory cells showed positive Ki-67 and P53 expression, while in the study by Afaf *et al.* [27], 32.7 % of these cases had a positive expression of P53.

Ki-67 and P53 were expressed in 92% of cases with infiltration of PMN cells, while in the study by Afaf *et al.* [27], 66.7 % of these cases had a positive expression of P53.

Furthermore, 92.3% of cases with atrophy had positive Ki-67 and P53 expression, while in the study by Afaf *et al.* [27], 26.1 % only of the cases had positive P53 expression.

Although they are few detected in this study, all cases with intestinal metaplasia showed positive P53 expression, and only half of them showed Ki-67 expression, while in Mahmoud Zadeh *et al.* [23] study 60%, 55% only of these cases had positive P53 and Ki-67 expression, respectively, while in Afaf *et al.* [27] study, 31.7% only of these cases had positive P53 expression. On the contrary to the results of this study, Forones *et al.* [30] found in their study that all samples with intestinal metaplasia had a negative expression of P53, and they supposed that the mutation of P53 occurs in the latter stages of gastric carcinogenesis. The explanation of the reason for the lack of the presence of the intestinal metaplasia in a large number of cases in

this research belongs to exclusion of negative cases of HP, and as it is known, this bacterium does not colonize in the intestinal metaplastic tissues.

Regarding the cases with dysplasia, again all of them showed positive expression of P53, while only 2/3 of them showed Ki-67 expression, while in Mahmoud Zadeh *et al.* [23] study 57.89%, 26.31 % only of these cases had positive expression, respectively.

Conclusions

Ki-67 and P53 expressions increase with chronicity of HP positive gastritis,

P53 expression is amplified when atrophy is present in HP-positive gastritis samples.

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