

An Immuno-Histochemical Assessment of Ki67, P53 Over-Expression in *Helicobacter Pylori* Positive Gastritis.

Karam Turath Tawfeeq (✉ h.aqeel@alnukhba.edu.iq)

University of Mosul College of Medicine

Elaf Abdulwahhab Hamdi

University of Mosul College of Medicine

Nadwa Subhi Al-azzo

University of Mosul College of Medicine

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Abstract

Background and Objective: *Helicobacter pylori* infection of the stomach is a common disease and the resulting changes from it are numerous and deserve to be in the focus of researchers' attention. The aim of this study is to assess the expression of mutant P53 protein and Ki-67 markers in patients with gastritis secondary to *Helicobacter pylori*.

Methods: Thirty samples with positive *Helicobacter pylori* gastritis were included in a retrospective study in Mosul / Iraq. The histological parameters were assessed using the Sydney system, then the expression of Ki67 and P53 expression were studied by immunohistochemical methods. The significance level was appointed at (0.05).

Results: Ki67 and P53 expression were found in 83.3% of the total cases. The study results show that 92% of positive Ki67, P53 cases had chronic inflammatory cell infiltration, polymorph nuclear cells infiltration, and atrophy. Whereas 96% of positive Ki67 cases had no metaplasia, 92% of the positive P53 cases had no metaplasia. The results also showed that only 16% of the positive Ki67 cases had dysplastic changes, and 24 % of the positive cases of P53 cases were showed dysplasia. moreover, whenever P53 was negative; there is neither metaplasia nor dysplasia in the tissue, this does not apply to Ki67 negative cases.

Conclusions: Ki67, P53 expressions increase with chronicity of *Helicobacter pylori*-positive gastritis, P53 expression is amplified when atrophy is present in these samples

1. 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 affect about 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 in order 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 commonest 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 nonsteroidal anti-inflammatory drugs, smoking, narcotics, alcohol and autoimmune diseases ⁽⁴⁻⁸⁾.

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 like glands of intestinal tissues and called intestinal metaplasia which is found in end-stage disease and are considered as a premalignant lesions ^(9, 10).

The long-term presence of HP leads to persistent damage and hyper proliferation of the lining epithelium of stomach, that will lead to an increase in the occurrence of mutations and 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 occur 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 on 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⁽¹⁷⁻²⁰⁾. The previous findings have shown that the expressions of P53 and Ki67 were associated with each other. However, these markers alone had no diagnostic value^(21,22).

Aim: The aim of this study is 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 Sydney system .

2. Materials And Methods:

Study setting & 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 collected from Nineveh governorate private laboratories and teaching hospitals, then processed in all procedures needed together with data analysis from October 2020 through April 2021. Clinical data was collected including the age, 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, metaplasia, in which the cases were divided into 4 grades as follows: - 0: not present, 1: mild, 2: moderate, 3: severe. Dysplasia assessment was added to Sydney system.

After collecting 30 cases of HP positive gastritis, the Ki67 and P53 over-expression were studied by immunohistochemical method using Dako monoclonal antibody for Ki67 and P53 Protein.

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%; 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 positive expression for that marker and less than 4 were considered negative expression for that marker⁽²⁴⁾.

Statistical analysis

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

3. Results:

Marker expression

In this study, 30 patients with HP-positive gastritis were recovered, all samples were antral biopsies, Ki67 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 Ki67 concordance with P53 was statistically in fair agreement ($\kappa = 0.267$). (Figure -1)

Patient's age & sex

The age of the patients ranged from 14 to 84 years with mean 46 years \pm 17.2 (Table 1,2), the largest group of Ki67 positive cases (36%) were in the age group (42-55) years, again the largest group of P53 positive cases (40%) also were in the age group (42-55) years, with significant p-values (0.039) and (0.019), respectively.

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

Histological examination

About 2/3 of cases with positive Ki67 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. Although 96% of them did not have metaplasia with significant p-value (0.013). On the other hand, more than half of the negative Ki67 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).

Lastly, whilst 16% of positive Ki67 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).

4. Discussion:

Many researchers have studied the expression of Ki67 & P53 in both positive and negative HP gastritis, gastric ulcer, and cancer. In this study, the expression of Ki67 & P53 were assessed only in positive HP gastritis and the expression of Ki67 & P53 were detected in 83.3% of cases, a higher percentage more than many other studies, such as: Azza et al ⁽²⁵⁾ study in which Ki67 & P53 expression were 54.5%, 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 size of the cases taken by the studies, different genetic background, 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 Ki67 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 14-84 years with mean 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. Ki67 & P53 expressions in this study were significantly detected more in age group (42-55) years, also the expression of both Ki67 & P53 were 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 Ki67 & P53 expression, while in the study by Afaf et al ⁽²⁷⁾ 32.7% of these cases had positive expression of P53.

Ki67 & 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 positive expression of P53.

Furthermore, 92.3% of cases with atrophy had positive Ki67 & 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 Ki67 expression, while in Mahmoudzadeh et al ⁽²³⁾ study 60%, 55% only of these cases had positive P53, Ki67 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 negative expression

of P53, and they supposed that the mutation of P53 occur in latter stages of gastric carcinogenesis. The explanation of the reason for the lack of the presence of the intestinal metaplasia in large number of cases in this research is belong 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 Ki67 expression, while in Mahmoudzadeh et al⁽²³⁾ study 57.89%, 26.31% only of these cases had positive expression respectively.

5. Conclusions:

Ki67, P53 expressions increase with chronicity of HP positive gastritis,

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

Declarations:

6. Acknowledgment:

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7. Conflict of interest:

The authors declare that they have no conflict of interest.

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Tables

(Table -1- Age distribution with Ki67 expression)

Age groups (years)	No.	Ki67 +ve No. (%)	Ki67 -ve No. (%)	p-value
< 28	4	4 (16.0)	0 (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)	

(Table -2- Age distribution with P53 expression)

Age groups (years)	No.	P53 +ve No. (%)	P53 -ve No. (%)	p-value
< 28	4	4 (16.0)	0 (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)	

(Table -3- Sydney system with Ki67 expression)

Sydney system		No.	Ki67 +ve No. (%)	Ki67 -ve No. (%)	p-value
Chronic Inflammation	0	1	0 (0.0)	1 (20.0)	0.014
	1	5	3 (12.0)	2 (40.0)	
	2	9	9 (36.0)	0 (0.0)	
	3	15	13 (52.0)	2 (40.0)	
	Total	30	25 (100.0)	5 (100.0)	
P.M.N.	0	5	2 (8.0)	3 (60.0)	0.227
	1	7	7 (28.0)	0 (0.0)	
	2	12	10 (40.0)	2 (40.0)	
	3	6	6 (24.0)	0 (0.0)	
	Total	30	25 (100.0)	5 (100.0)	
Atrophy	0	4	1 (4.0)	3 (60.0)	0.131
	1	7	7 (28.0)	0 (0.0)	
	2	13	11 (44.0)	2 (40.0)	
	3	6	6 (24.0)	0 (0.0)	
	Total	30	25 (100.0)	5 (100.0)	
Metaplasia	0	28	24 (96.0)	4 (80.0)	0.013
	1	1	0 (0.0)	1 (20.0)	
	2	1	1 (4.0)	0 (0.0)	
	3	0	0 (0.0)	0 (0.0)	
	Total	30	25 (100.0)	5 (100.0)	

(Table -4- Sydney system with P53 expression)

Sydney system		No.	P53 +ve No. (%)	P53 -ve No. (%)	p-value
Chronic Inflammation	0	1	0 (0.0)	1 (20.0)	0.020
	1	5	2 (8.0)	3 (60.0)	
	2	9	8 (32.0)	1 (20.0)	
	3	15	15 (60.0)	0 (0.0)	
	Total	30	25 (100.0)	5 (100.0)	
P.M.N.	0	5	2 (8.0)	3 (60.0)	0.110
	1	7	6 (24.0)	1 (20.0)	
	2	12	11 (44.0)	1 (20.0)	
	3	6	6 (24.0)	0 (0.0)	
	Total	30	25 (100.0)	5 (100.0)	
Atrophy	0	4	1 (4.0)	3 (60.0)	0.078
	1	7	5 (20.0)	2 (40.0)	
	2	13	13 (52.0)	0 (0.0)	
	3	6	6 (24.0)	0 (0.0)	
	Total	30	25 (100.0)	5 (100.0)	
Metaplasia	0	28	23 (92.0)	5 (100.0)	0.034
	1	1	1 (4.0)	0 (0.0)	
	2	1	1 (4.0)	0 (0.0)	
	3	0	0 (0.0)	0 (0.0)	
	Total	30	25 (100.0)	5 (100.0)	

Figures

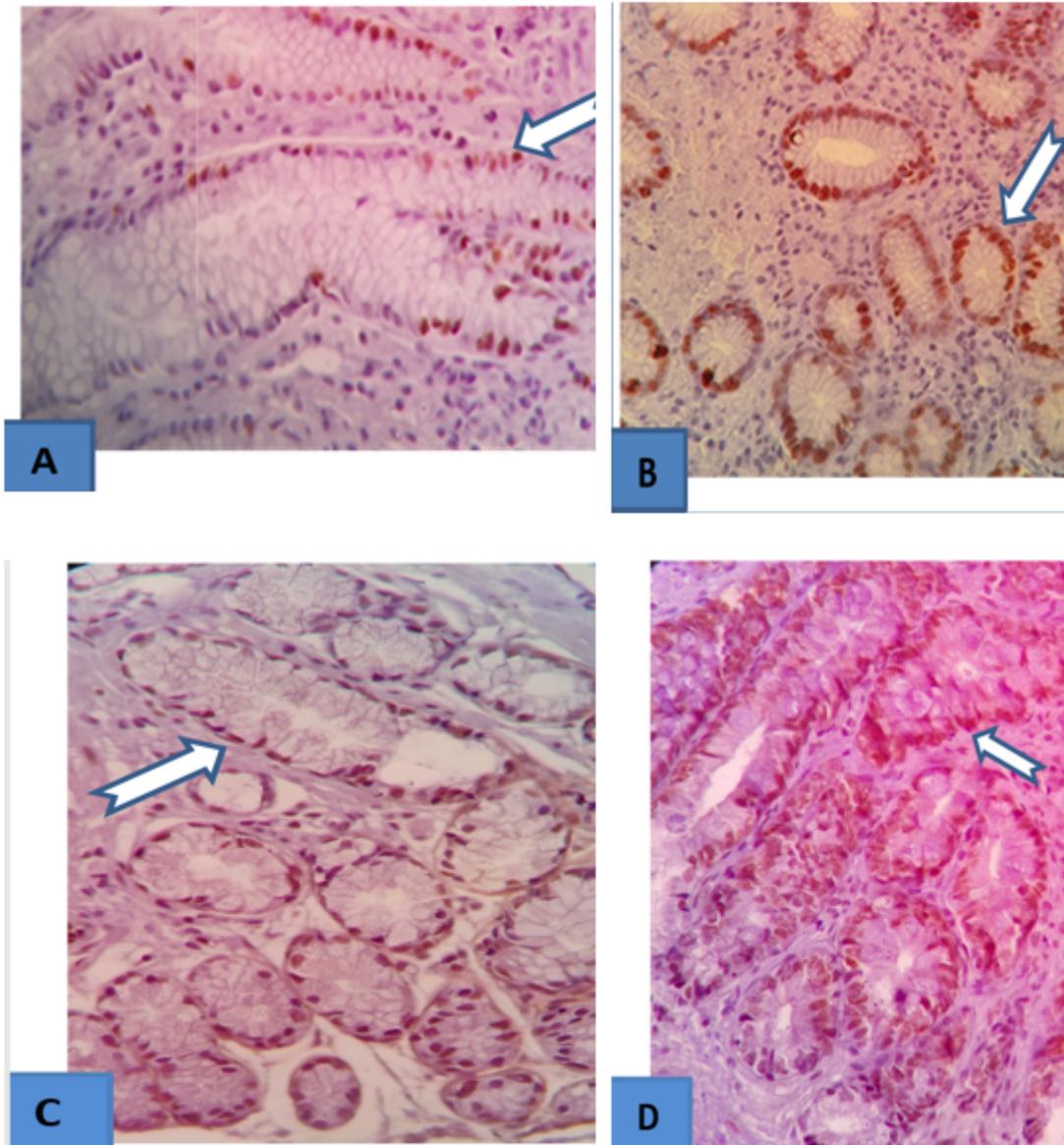


Figure 1

H. pylori positive gastritis with weak Ki-67 expression magnification-400. shows four cases with weak and strong expression of Ki67 and P53 expression in this figure (A) , strong Ki-67 expression (B); weak P53 expression (C) and strong P53 expression (D) appeared as brown colour (arrows) magnification-400.

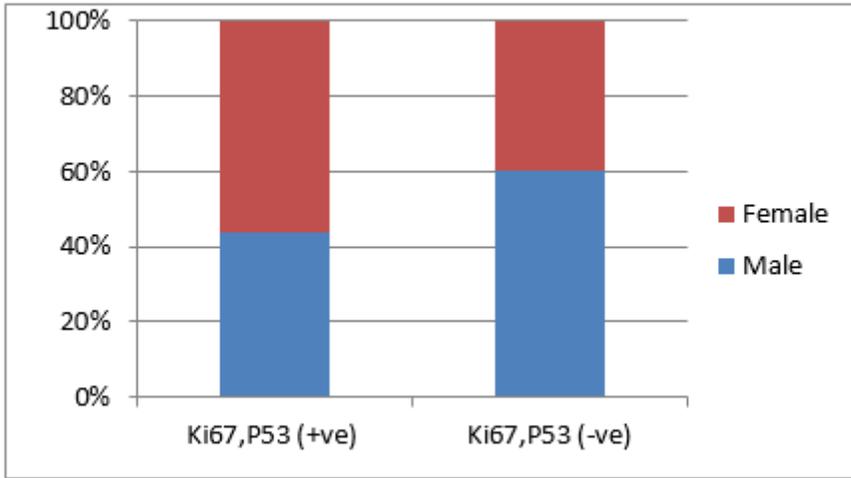


Figure 2

Sex distribution with Ki67, P53 expression, p-value (0.409)

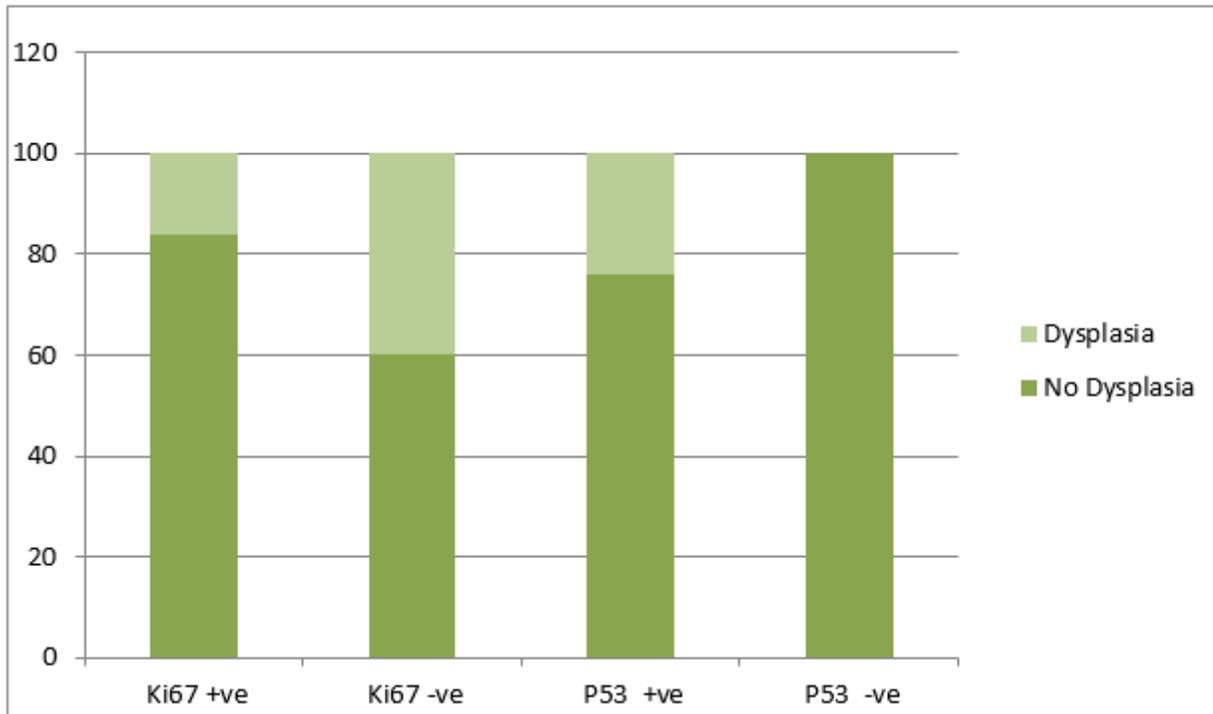


Figure 3

Dysplasia with Ki67 expression: p-value (0.074), P53 expression: p-value (0.467)