Original Article

Expression of cytokeratin 7/20 and Ki67 in *Helicobacter pylori*-associated gastritis and intestinal metaplasia

Ava T Ismael[1], Rafal A Abdulhameed[2], Jalal A Jalal[3], Rawaz D Tawfeeq[4], Aram Ommar[5], Aveen Jalal[6]

[1] Department of Pathology, College of Pharmacy, Hawler Medical University, Erbil, Iraq
[2] Department of Basic Sciences/Pathology, College of Medicine, Hawler Medical University, Erbil, Iraq
[4] Pathophysiology, Department of Clinical Analysis, College of Pharmacy, Hawler Medical University, Erbil, Iraq
[5] Department of Clinical Pharmacy, College of Pharmacy, Hawler Medical University, Erbil, Iraq
[6] School of Pharmacy, Nottingham University, Nottingham, United Kingdom

Abstract
Introduction: Cytokeratins (CKs) have been associated with precancerous and cancerous gastric lesions in patients with *Helicobacter pylori*-associated chronic gastritis, making them useful for diagnosing epithelial tumors.
Methodology: A retrospective study was conducted utilizing 200 formalin-fixed paraffin-embedded gastric biopsy samples collected from the lesser curvature of the stomach. Samples from the control group, patients with *H. pylori* infection, and patients with *H. pylori*-associated gastritis, with complete and incomplete intestinal metaplasia (IM) were immunostained. Monoclonal antibodies were utilized to determine the expression of CK7, CK20, and Ki-67.
Results: Patients infected with *H. pylori* had strong CK20 expression on the surface, and weak CK7 expression on the surface and deep glands; while non-specific chronic gastritis patients had weak focal CK7 expression and strong CK20 expression. The normal gastric mucosa of patients in the control group had relatively weak CK7 expression, restricted to a few cells in the neck and deep glands. CK20 showed diffuse strong reactivity on the surface. On the other hand, patients with complete IM showed a CK7 staining pattern that was either negative or weakly focal on the surface and crypts associated with diffuse surface CK20 and focal crypt staining corresponding to gastric type IM. The Ki67 proliferating index was low (≤ 15%) in *H. pylori* infected patients, high (> 30%) in patients with incomplete IM, and intermediate (16–30%) in patients with complete IM.
Conclusions: These results indicate a significant link between the expressions of CK7/CK20 and Ki67 in patients afflicted with *H. pylori* and IM.

Introduction

Helicobacter pylori (*H. pylori*) is a helical, Gram-negative, microaerophilic, flagellated bacterium. It is capable of generating a biofilm and changing from a spiral shape to its coccoid form. It has a strong correlation with intestinal metaplasia (IM), precancerous gastric atrophy, and gastric inflammation [1,2]. *H. pylori* infections result in a variety of inflammatory processes such as the infiltration of macrophages, neutrophils, regulatory T-cells, and natural killer cells which have a substantial impact on the gastric milieu [3]. *H. pylori*-related chronic gastritis causes numerous structural alterations in the stomach epithelium, including variations in the production of cytokeratins (CKs), regardless of the fact that more than 80% of infected individuals are asymptomatic [4,5].

CKs are a family of key cytoskeleton proteins and are intermediate filament subunits that are found in epithelial cells. They are genetically predetermined, and the level of epithelial differentiation influences expression [6]. Almost all simple epithelia solely express cytokeratin 7 (CK7) — a 54 kD (intermediate-sized and basic) polypeptide. It is present in normal epithelia including those of the bladder, bile ducts, cervix, breasts, and lungs; but is absent from gastrointestinal and squamous epithelia [7]. The gastric surface foveolar epithelium, intestinal villi, and crypt epithelium all contain cytokeratin 20 (CK20), which is an acidic protein of intermediate size. Inflammatory, preneoplastic, and neoplastic illnesses of the gastrointestinal tract have been associated with a variety of alterations in the expression and distribution of CKs within the gastrointestinal tract [6].

Both CK7 and CK20 display a constrained pattern of expression. CK7 and CK20 reactivity of the gastric mucosa is related to the various stages of cell

development. Based on previous studies, CK7 is transiently neoexpressed in metaplastic and neoplastic gastric epithelial cells. CK7 is expressed in the fetus but is generally missing in healthy adults [5]. The development of metaplasia and neoplasia may consequently be accompanied by a fetal-like dedifferentiation of cellular phenotypes, as suggested by CK7 neoexpression in the stomach [8].

The aim of this study was to examine the expression of CK 7/20 and the Ki67 proliferative activity index in the altered stomach epithelium of individuals with chronic gastritis and *H. pylori* infections, both of which are linked to IM. A study of this nature is important because it will aid future research aimed at early detection of gastric cancers. Furthermore, it strengthens and supports previous exploratory studies in this field.

Methodology

A retrospective study was conducted from January 2016 until January 2017 on 200 formalin-fixed, paraffin-embedded specimens of gastric biopsies taken from the lessor curvature (corpus and antrum) of the stomach. These specimens were retrieved from the histopathology laboratory at Rizgary Teaching Hospital and private histopathology labs in Erbil situated in the Kurdistan Region of Iraq. The study was approved by the Ethics Committee at the College of Pharmacy in Hawler Medical University (approval no. HMU-PH-EC No: 191105/100).

Figure 1. Flow chart showing sample collection strategy for the study.

IM: intestinal metaplasia.

Demographic data and clinical information were obtained from patient records. This information included the patients' age, gender, and the location of the extracted biopsy. The modified Sydney system was used to assess the type of gastritis, the level of neutrophil infiltration, lamina propria mononuclear cell infiltration, IM, and glandular atrophy [9]. Biopsies that revealed IM were categorized in accordance with earlier suggestions made by Shen *et al.* in 2002 [10]. The slides were reviewed by two experienced pathologists. Figure 1 describes the flow of the data collection processes.

The control group consisted of patients with no histological changes in the gastric mucosa. Twenty-nine cases were excluded from the study via the criteria listed in Figure 1. Therefore, the total number of subjects for the study was 171 patients. These included 71 men (41.5%) and 100 women (58.5%). Furthermore, 78 of the patients (45.6%) were above 40 years old, whilst 93 patients (54.4%) were under 40 years old. An esophagogastroduodenoscopy was conducted on all samples using biopsies obtained from the lessor curvature (corpus and antrum). Rapid urease testing, histological analysis, immunohistochemistry, and Giemsa stain were used to identify the presence of *H pylori* (Figure 2). Histology and at least one other diagnostic technique had to be positive for a patient to be classified as *H pylori* positive.

Histological evidence of IM was documented in each case and defined as being either type I, II, or III. Alcian blue (pH 2.5) analysis utilizing high iron diamine (AB-HID) stained sections, which revealed the presence of acid mucin within goblet cells, was used to determine the presence of IM. Goblet cells alternating with absorptive enterocytes were found in type I or full IM. Columnar cells would be alternating with goblet cells in type II and type III, or incomplete IM. The columnar cells containing sialomucins were primarily found in type II IM, and were identified with a blue stain. Alternatively, they contained sulphomucins found in type III incomplete IM which was identified via a black stain.

Only 30 cases with sufficient areas of IM could be studied for both AB-HID staining. In this process, four sections that were 4 mm thick were taken from each block of paraffin embedded tissue. The sections were put on an ordinary slide for hematoxylin and eosin (H&E) staining in order to confirm the diagnosis of *H. pylori*-positive gastritis; other sections were put on the charged slides for immunohistochemistry (IHC) staining. Only samples permitting measurements of the complete mucosal thickness were chosen for the
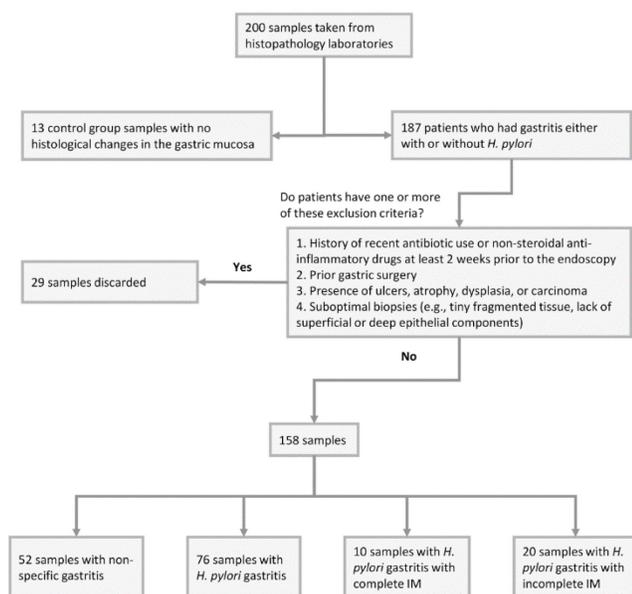
immunohistochemical research on consecutive sections of the selected stomach biopsy for each patient in order to detect the expression of CK7 and CK20, and the Ki67 proliferative index. *H. pylori* positive gastritis identified via different staining techniques (H&E, Giemsa, and IHC) is presented in Figure 2.

Immunohistochemistry

A standard three-step streptavidin-biotin complex staining procedure was used on the charged slides after the sections were sliced. The slides were rehydrated in 100%, 96%, and 70% ethanol, and water after being deparaffinized for 3 to 10 minutes in xylol. Endogenous peroxidase was inhibited in a mixture of ethanol with 3% hydrogen peroxide for 10 minutes. After the slides were rinsed in water, antigen retrieval was done via heat treatment by microwaving the slides for 10 minutes in a citrate-buffered solution (10 mmol/L, pH 6.0). Afterwards, the slides were rinsed in phosphate-buffered saline (PBS).

The tissue sections were incubated for 1 hour at room temperature in 1.5% bovine serum albumin (BSA) to block non-specific antigen sites. The sections were stained with one of two monoclonal antibodies. The first one was CK7, which is a monoclonal mouse anti-human CK7 clone (OV-TL 12/30 Code M7018) supplied by the Danish company Dako (Dako, Santa Clara, United States). The second monoclonal antibody was CK20, which is a monoclonal mouse anti-human CK20 clone (Ks 20.8 code M7019) that was also obtained from the same manufacturer (Dako, Santa Clara, United States).

The sections were stained with Ki67 monoclonal antibodies which were manufactured in the United States by Dako (Santa Clara, United States). These antibodies were diluted (1:100) with 1% BSA. Ki67 was used to assess cell proliferation because it allows for an approximation of the cell population's growth fraction.

Immunohistochemical staining was performed using a streptavidin-biotin/horseradish peroxidase detection kit in line with the manufacturer's instructions. This kit was once again provided by Dako (Dako, Santa Clara United States). After gently rinsing the sections with distilled water, they were counterstained with hematoxylin. Positive and negative controls were included in each IHC run. The positive control samples were composed of normal colonic epithelium, and the majority of primary tumors with an adjacent normal epithelium served as internal control. A duplicate section stained with distilled water rather than the primary antibody served as the negative control.

Evaluation of the IHC was done by two independent, blinded pathologists who had no prior knowledge of the clinical and endoscopic data. These pathologists described the mucosal distribution in the surface epithelium, foveolar region, glandular necks, and deep glands of the slides. Furthermore, they also documented the intensity of cytoplasmic staining which was divided into one of three categories (no staining, weak, moderate-strong staining). The expression pattern of each of the analyzed CK samples were categorized into being either focal or diffuse. The percentages of immunoreactive cells in each mucosal compartment were also semi-quantitatively evaluated and ranked for CK. These results were subdivided into one of three groups (< 10% (+), 10–20% (++), and > 20% (+++)) [6].

Cell proliferation was evaluated in all the groups and defined as the percentage of cells with positively immunostained nuclei. This consisted of counting at least 1000 cells in 10 different consecutive high-power fields (400× magnification). The Ki67's proliferation index was divided into three groups: low (Ki67: 15%),

Figure 2. *H. pylori* positive gastritis observed with different staining techniques. **A.** hematoxylin and eosin (H&E); **B.** Giemsa; **C.** immunohistochemistry (IHC).
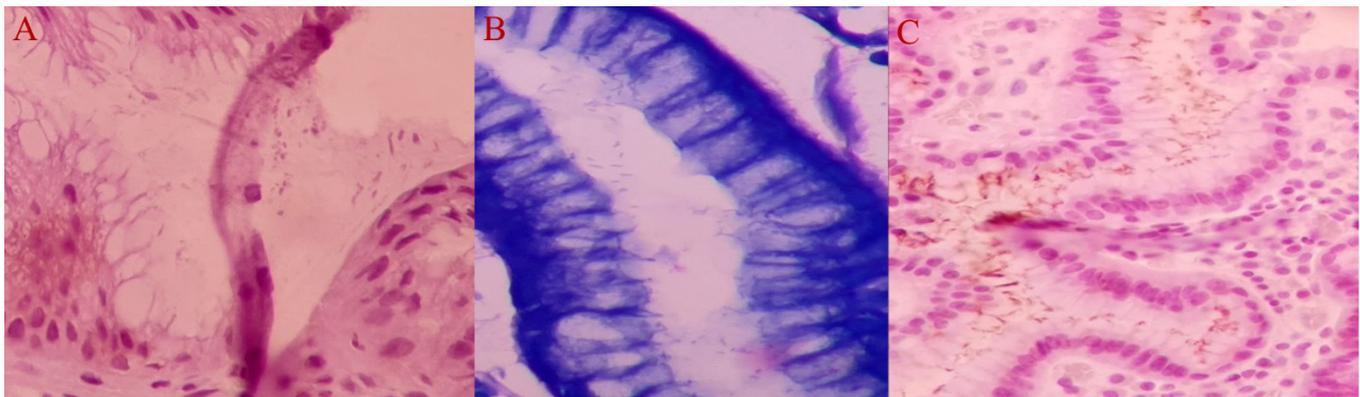
intermediate (Ki67: 16–30%), and high (Ki67 > 30%) [11].

Statistical analysis

All patient variables were recorded in an Excel spreadsheet. The data were analyzed using a Chi-square independence test, and contingency tables were generated using the Statistical Package for the Social Sciences (SPSS) program version 26. Chi square tests for 2×3, 3×3, or 3×4 contingency tables did not support calculations when a cell value of zero occurred. As a result, the Graph Pad Software version 10 was used to do a goodness-of-fit analysis (differences between observed and predicted cases). If the *p*-value was less than 0.05, the data was determined to be statistically significant.

Results

The groups in the study exhibited different patterns of expression for both CK7 and CK20 in different regions. Notable differences were also observed in Ki67 proliferation (Tables 1 and 2)

In the control group, there was an absence of CK7 expression on the surface, foveolar and glandular neck, with weak focal staining in the deep glands (23.08%). On the other hand, CK20 showed strong diffuse reactivity (76.92%) and weak focal reactivity (23.08%) on the surface and foveolar epithelium, while it was negative in the deep glands (Figure 3). Furthermore, the Ki67 proliferating index was low (Ki67 ≤ 15%) as can be seen in Figure 4.

Table 1. Expression of cytokeratin 7.

| CK7	No. of controls (%)	No. of chronic gastritis (%)	No. of *H. pylori* +ve (%)			Total	*p* value
			Total	With incomplete IM	With complete IM		
Surface epithelium							
Diffuse	(0) -ve	(0) -ve	(0) -ve	3 (15) +++	(0) -ve	3	0.01
Focal	(0) -ve	2 (3.8) +	6 (7.9) +	4 (20) +++	(0) -ve	12	0.02
No stain	13 (100)	50 (96.2)	70 (92.1)	13 (65)	10 (100)	156	0.01
Foveolar epithelium							
Diffuse	(0) -ve	(0) -ve	(0) -ve	3 (15) +++	(0) -ve	3	0.01
Focal	(0) -ve	3 (5.8) +	7 (9.2) +	4 (20) +++	(0) -ve	14	0.01
No stain	13 (100)	49 (94.2)	69 (90.8)	13 (65)	10 (100)	154	0.01
Glandular neck							
Diffuse	(0) -ve	(0) -ve	14 (18.42) ++	3 (15) +++	(0) -ve	17	0.01
Focal	(0) -ve	5 (9.6) +	12 (15.79) ++	12 (60) ++	2 (20) +	31	0.01
No Stain	13 (100)	47 (90.4)	50 (65.79)	5 (25)	8 (80)	123	0.01
Deep glands							
Diffuse	(0) -ve	(0) -ve	12 (15.79) ++	5 (25) +++	(0) -ve	17	0.01
Focal	3 (23.08) +	3 (5.77) +	20 (26.32) ++	12 (60) ++	3 (30) +	41	0.01
No stain	10 (76.92)	49 (94.23)	44 (57.89)	3 (15)	7 (70)	113	0.01
Total	13 (7.60)	52 (30.41)	76 (44.44)	20 (11.70)	10 (5.84)	171	

The results revealed that the *p* values calculated from the Chi-square contingency tables to represent the associations among expressions of CK 7 with various sites were as follows: for surface epithelium: $\chi^2 = 248.04$, $p = 0.0001$; foveolar epithelium: $\chi^2 = 248.67$, *p* value = 0.0001; glandular neck: $\chi^2 = 113.56$, *p* value = 0.0001; and deep glands: $\chi^2 = 85.09$, *p* value = 0.0001. These results indicate a statistically significant difference in CK7 expression in various sites between the different groups studied. The percentages of immunoreactive cells found in each compartment were quantitatively divided into one of three groups (< 10% (+): 10–20% (++): and > 20% (+++)). CK: cytokeratin; IM: intestinal metaplasia.

Table 2. Expression of cytokeratin 20.

CK2	No. of controls (%)	No. of Chronic Gastritis (%)	No. of *H. Pylori* +ve (%)			Total	*p* value
			Total	With incomplete IM	With complete IM		
Surface epithelium							
Diffuse	10 (76.92) +++	40 (76.92) +++	26 (34.21) +++	12 (60) +++	9 (90) +++	97	0.001
Focal	3 (23.08) +	12 (23.08) ++	29 (28.16) ++	8 (40) +++	1 (10) ++	53	0.001
No Stain	0 -ve	0 -ve	21 (27.63)	0 -ve	0 -ve	21	No significance
Foveolar Epithelium							
Diffuse	10 (76.92) +++	38 (73.08) +++	20 (26.32) +++	10(50) +++	8(80) ++	86	0.001
Focal	3 (23.08) +	14 (26.92) ++	26 (34.21) +	6(30) +++	1(10) ++	50	0.001
No Stain	0 -ve	0 -ve	30 (39.47)	4(20)	1(10)	35	0.001
Deep glands							
Diffuse	0 -ve	0 -ve	0 -ve	0 -ve	0 -ve	0	No significance
Focal	0 -ve	12 (23.8) +	10 (13.16) +	14(70) ++	2(20) ++	38	0.001
No Stain	13 (100)	40 (76.92)	66 (86.84)	6(30)	8(80)	113	0.001
Total	13 (7.60)	52 (30.41)	76 (44.44)	20 (11.70)	10(5.84)	171	

The *p* values were calculated from the contingency table of Chi-squares and represent the associations among expressions of CK 20 with various sites as follows: surface epithelium: $\chi^2 = 51.09$, *p* value = 0.0001; foveolar epithelium: $\chi^2 = 24.11$, *p* value = 0.0001; and deep glands: $\chi^2 = 160.04$, *p* value = 0.0001. These results revealed highly significant associations among expressions of CK 20 with various sites. The percentages of immunoreactive cells found in each compartment were quantitatively divided into one of three groups (< 10% (+), 10–20% (++), and > 20% (+++)).

Figure 3. CK7 pattern in the control group (A) and CG group (B). CK20 pattern in the control group (C) and CG group (D). These images were obtained with a 100× microscope.
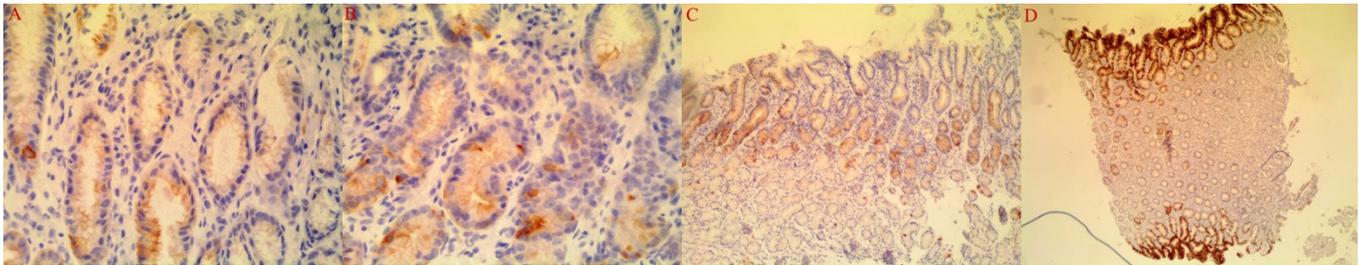
In patients with non-specific gastritis, CK7 showed a weak focal staining pattern on the surface (3.8%), foveolar (5.8%), glandular neck (9.6%), and in the deep glands (5.77%). In contrast, CK20 showed strong diffuse (76.92%) and moderate focal (23.08%) staining on the surface epithelium; as well as a strong diffuse (73.08%) staining and moderate focal (26.92%) staining in the foveolar epithelium and deep glands. Furthermore, there was a weak focal pattern in the deep glands (23.8%) as can be seen in Figure 3. The Ki67 proliferating index was low (Ki67 ≤ 15%) (Figure 4).

In patients with *H. pylori* gastritis, three patterns for CK7/20 were observed. The predominant CK7 pattern was mostly an absence of staining on the surface (92.1%), foveolar epithelium (90.8%), glandular neck (65.79%), and deep glands (57.89%). The second most common pattern was an absence of staining on the surface and foveolar epithelium, while it showed moderate diffuse (18.42%) staining on the glandular neck and in the deep glands (15.79%). The third pattern showed weak focal (7.9%) staining on the surface and foveolar epithelium (9.2%); and moderate focal staining in the glandular neck (15.79%), and in deep glands (26.32%).

A proportion of samples had a strong diffuse (34.21%) CK7 pattern on the surface and foveolar epithelium (26.32%), while it was absent in the deep glands. The second pattern was moderate focal staining on the surface epithelium (28.16%) and weak focal staining on the foveolar epithelium (34.21%) and in the deep glands (13.16%). The third pattern was an absence

of staining on the surface (27.63%), foveolar epithelium (39.47%), and the deep glands (86.84%) (Figure 5).

The type of immunostaining was divided into three patterns based on the type of the IM and whether it was incomplete or complete IM. In the case of incomplete IM, the pattern for CK7 on surface epithelium of most of the samples showed an absence of staining (65%), followed by either having focal (20%) or diffuse (15%)

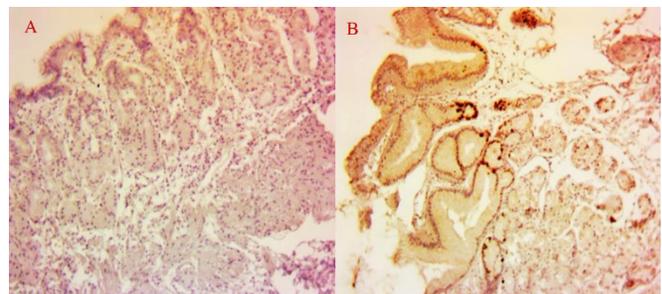**Figure 4.** Ki67 proliferating index in the control group (A) and CG group (B) under a microscope (100×).

Figure 5. CK7 (A) and CK20 (B) expression under a 100x microscope in a patient with H. pylori gastritis 100×
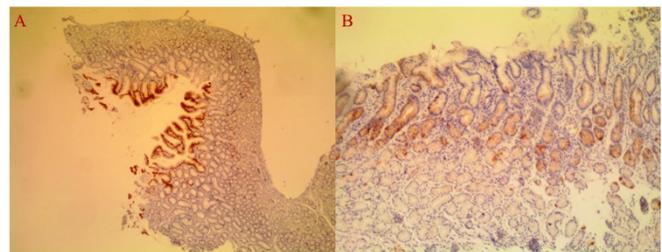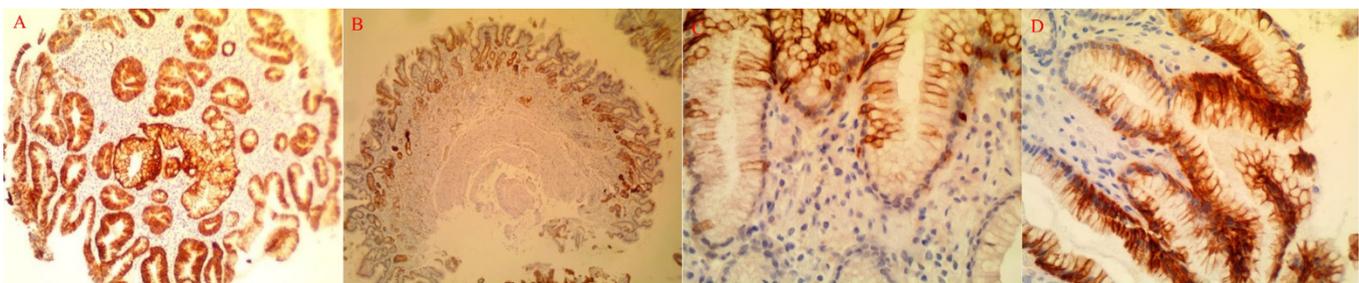

Figure 6. CK7 pattern in incomplete IM (A, C, and D), and CK20 pattern (B), observed with a 100× microscope.

staining pattern. In the case of the foveolar epithelium, most samples had an absence (65%) of staining, while a minority had either diffuse (15%) or focal (20%) staining. In the glandular neck, the samples had either focal (60%), diffuse (15%), or no staining (25%) present. In the deep glands, the samples displayed focal (60%) staining mostly followed by either diffuse (25%) staining or no stain (15%).

The predominant pattern for CK20 staining on the surface of the samples was a strong diffuse (60%) staining followed by focal staining (40%) (Figure 6). None of the samples displayed an absence of staining on the surface epithelium. Furthermore, the samples displayed either a diffuse (50%), focal (30%), or no staining pattern (20%) on the foveolar epithelium. In the deep glands, the majority of samples displayed a focal staining pattern (70%) followed by an absence of staining (30%); while none of the samples displayed a diffuse staining pattern. The Ki67 proliferating index was high (Ki67 > 30%) (Figure 8).

The pathologists noted that in all the samples from IM, there was an absence of CK7 in the surface and foveolar epithelium, while within the glandular neck most samples had no stain present (80%), and a minority displayed a weak focal staining pattern (20%). Furthermore, most samples had an absence of CK7 (70%) in the deep glands and a minority displayed a weak focal staining pattern (30%). Regarding the presence of CK20, most of the slides displayed a strong diffuse staining pattern (90%) and a minority displayed a moderately focal staining (10%) pattern in the surface epithelium. Figure 7 provides a visual depiction of the pattern of CK7/CK20 expression in patients with IM. Furthermore, most samples displayed a moderately diffuse pattern in the foveolar epithelium (90%) and an absence of staining in the deep glands (80%; Figure 6). The Ki67 proliferating index was intermediate (Ki67: 16–30%; Figure 8).

Figure 7. CK7 (A) and CK20 (B) pattern in a patient with complete IM, observed with a 100× microscope.
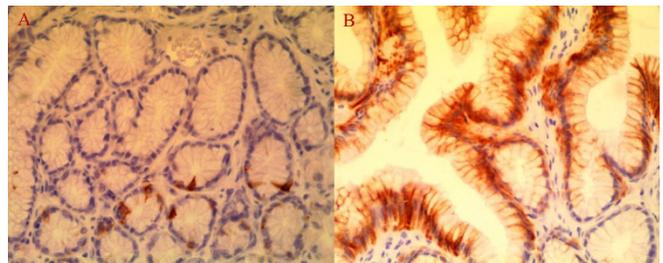
Discussion

Chronic gastritis is one of the risk factors for stomach cancer when associated with *H. pylori* infection. *H. pylori* is present in 75% of the aforementioned carcinoma cases, and has been shown in previous experimental and epidemiological research to play a significant role in the development of stomach carcinogenesis [12]. In countries such as Japan where the prevalence of stomach cancers is high, treating *H. pylori* is part of the medical strategy [13].

The inflammatory processes associated with chronic gastritis induce many histopathological changes in the gastric wall that lead to a continuous synthesis of cytokines, that in turn activate the immune system. Activation of the immune system leads to the generation of oxidative free radicals that may damage the DNA of host cells [14]. As a result, the onset of mucosal aberrant apoptosis is closely related to *H. pylori*-induced gastritis. It may also lead to atrophy, IM, and dysplasia which are pre-neoplastic gastric lesions that promote carcinogenesis and subsequently metastasis [15,16].

This study investigated the pattern of CK7/20 expression in patients infected with *H. pylori* associated gastritis. The biopsies were obtained from the lessor curve of the stomach. The studied individuals experienced *H. pylori*-related stomach inflammation from the cardia to the corpus/antrum.

Figure 8. Ki67 proliferating index in a patient with H. pylori gastritis (A), in a patient with complete IM (B), and in a patient with incomplete IM (C), observed with a 100× microscope.
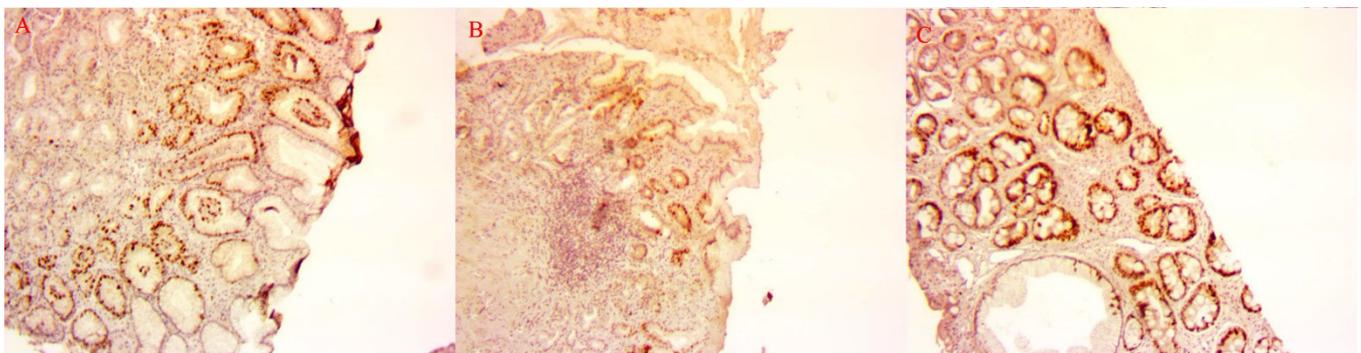
Based on our findings, the expression of CK7 in the control group's normal stomach mucosa was weak and limited to a few cells at the neck and deep glands. Furthermore, there was a strong diffuse CK20 expression on the surface, while it was negative in the deep glands. In contrast, patients with chronic non-specific gastritis showed a weak focal staining pattern of CK7 with a strong CK20 staining pattern on the surface, corresponding to a gastric type pattern of CK7/20.

In patients infected with *H. pylori*, the predominant profile was an absence of staining in the surface epithelium, foveolar epithelium, glandular neck, and deep glands with a strong diffuse CK20 pattern on the surface which was absent in the deep glands. Another uncommon pattern of CK7 was a weakly positive focal distribution on the surface and foveolar epithelium, as well as a moderately diffuse or moderate focal staining in the glandular neck and deep glands. This moderate reactivity of CK7 was observed where the glandular neck and deep glands showed inflammatory infiltration suggesting a neoexpression or dedifferentiation of CK7 in this region of the stomach. This highlights the ability of the *H. pylori*-infected mucosa to proliferate or regenerate. Cohen *et al.* reported that infection with *H. pylori* causes structural changes in the gastric epithelium that may lead to variations in the expression of CKs, and referred to these changes as early sequelae of *H. pylori*-associated gastritis [17,18].

Based on previous studies, IM is classified into IM type I, type II, and type III. IM type I (Barrett's type) is characterized by a significant diffuse CK7 staining in both the superficial and deep glands, as well as a strong superficial expression of CK20. IM type II (gastric type) is characterized by negative or weak focal CK7 staining on the surface and crypts. Furthermore, it is also associated with diffuse surface CK20 and focal crypt staining. IM type III is distinguished by prominent localized CK7 staining on the surface and crypt epithelium. It is also usually linked with crypt CK20 staining, both diffuse and localized [19]. Our results revealed that the predominant pattern is type II IM. The other two patterns (type I and III IM) were less common, and patients with complete IM displayed type II IM.

Our findings are consistent with those of Kirchner *et al*., who observed CK7 expression in patients with incomplete metaplasia as opposed to complete metaplasia, which was mostly CK7 negative. The researchers found that CK7 was positive in less developed, partial IM; but negative in differentiated, complete IM [20].

Shen *et al.* explained the difference between incomplete and complete IM based on a less differentiated versus well-differentiated hypothesis and found variations in CK expression in IM [10]. For example, patchy superficial and deep glands were stained with CK20, while the immunostaining of CK7 in deep glands was patchy. On the contrary, in areas with incomplete metaplasia there was a strong CK20 staining found in the superficial mucosa while there was an absence of CK7 expression in areas with complete IM. Thus, the presence of CK7 in areas of IM indicated an immature or incomplete gastrointestinal phenotype. However, CK20 expression was more unique for a more complete, mature, small-intestinal IM phenotype.

The Ki67 protein, which was used to analyze cell proliferation, made it possible to identify an approximation of the cell population growth fraction. It was detected in the germinative area of the gastric glands. Patients with *H. pylori* gastritis and patients with incomplete IM had a larger percentage of positive cells than the control group. The findings show that patients actively infected with *H. pylori* have increased mucosal epithelial cell proliferation fraction. This suggests the presence of high regenerative activity and is consistent with the interpretation of a hyper-proliferative state based on the CK7 results in this investigation. This is also consistent with the concept of a hyper-proliferative disease characterized by upward extension at the proliferative zone [21,22]. Verheijen *et al.* reported that Ki67 expression was higher in normal and neoplastic tissues as the cell cycle progressed [23]. Other investigations reported a greater cell proliferation index expression in chronic gastritis, which was strongly attributable to the presence of *H. pylori*, and is consistent with the findings of this research [24].

In their work, Kirchner *et al.* came to the conclusion that CK7 positivity in *H. pylori*-infected areas most likely represented the hyperproliferative state of the inflamed mucosa [20]. This observation could be the result of the *H. pylori* infection expanding the proliferative compartment within the gastric glands' germinative area. Consequently, the findings showed that patients who have *H. pylori* infection are more likely to have an enhanced mucosal epithelial cell growth percentage, which most likely indicates regeneration activity in the area.

Our study showed differences in CK7 and CK20 expression in patients who were *H. pylori* positive and had incomplete IM. These differences were attributed to the anatomical location of the biopsy since the studied samples were obtained from the lessor curvature of the stomach. Distal stomach biopsies were

far less likely to reveal a Barrett's type pattern of incomplete IM, which was linked to an elevated risk of cancer and was seen as a premalignant lesion in the distal stomach. Similar findings indicated that esophageal adenocarcinoma and inadequate IM were related.

A semi-quantitative assessment was also implicated in the differences in the CK staining pattern. However, global studies have reported variable results due to inter-observer variability, tissue fixation variances, and IHC differences (e.g. antigen retrieval methods) [25].

Conclusions

CK7 and CK20 can be instrumental in understanding the processes involved in the genesis and progression of the metaplasia-dysplasia-carcinoma sequence. As a result, detection of CK7 and CK20 is highly valuable valuable in the diagnosis of precancerous diseases [26].

References

1. Rajilic-Stojanovic M, Figueiredo C, Smet A, Hansen R, Kupcinskas J, Rokkas T, Andersen L, Machado JC, Ianiro G, Gasbarrini A, Leja M, Gisbert JP, Hold GL (2020) Systematic review: gastric microbiota in health and disease. Aliment Pharmacol Ther 51: 582–602. doi: 10.1111/apt.15650.
2. Sung JJY, Coker OO, Chu E, Szeto CH, Luk STY, Lau HCH, Yu J (2020) Gastric microbes associated with gastric inflammation, atrophy and intestinal metaplasia 1 year after *Helicobacter pylori* eradication. Gut 69: 1572–1581. doi: 10.1136/gutjnl-2019-319826.
3. Baj J, Brzozowska K, Forma A, Maani A, Sitarz E, Portincasa P (2020) Immunological aspects of the tumor microenvironment and epithelial-mesenchymal transition in gastric carcinogenesis. Int J Mol Sci 21: 2544. doi: 10.3390/ijms21072544.
4. Chojnacki C, Popławski T, Błońska A, Błasiak J, Romanowski M, Chojnacki J (2019) Expression of tryptophan hydroxylase in gastric mucosa in symptomatic and asymptomatic *Helicobacter pylori* infection. Arch Med Sci 15: 416–423. doi: 10.5114/aoms.2018.76928.
5. Kirchner T, Müller S, Hattori T, Mukaisyo K, Papadopoulos T, Brabletz T, Jung A (2001) Metaplasia, intraepithelial neoplasia and early cancer of the stomach are related to dedifferentiated epithelial cells defined by cytokeratin-7 expression in gastritis. Virchows Arch 439: 512–522. doi: 10.1007/s004280100477.
6. Todorovic V, Sokic-Milutinovic A, Drndarevic N, Micev M, Mitrovic O, Nikolic I, Wex T, Milosavljevic T, Malfertheiner P (2006) Expression of cytokeratins in *Helicobacter pylori*-associated chronic gastritis of adult patients infected with cagA+ strains: an immunohistochemical study. World J Gastroenterol 12: 1865–1873. doi: 10.3748/wjg.v12.i12.1865.
7. Chu PG, Weiss LM (2002) Keratin expression in human tissues and neoplasms. Histopathology 40: 403–439. doi: 10.1046/j.1365-2559.2002.01387.x.
8. Nobusawa A, Sano T, Negishi A, Yokoo S, Oyama T (2014) Immunohistochemical staining patterns of cytokeratins 13, 14, and 17 in oral epithelial dysplasia including orthokeratotic dysplasia. Pathol Int 64: 20–27. doi: 10.1111/pin.12125.
9. Şenocak Taşçı E, Akbaş T (2020) The relationship between the Sydney classification and the first-line treatment efficacy in *Helicobacter*-associated gastritis. Med Princ Pract 29: 551–557. doi: 10.1159/000508248.
10. Shen B, Ormsby AH, Shen C, Dumot JA, Shao YW, Bevins CL, Gramlich TL (2002) Cytokeratin expression patterns in noncardia, intestinal metaplasia-associated gastric adenocarcinoma: implication for the evaluation of intestinal metaplasia and tumors at the esophagogastric junction. Cancer 94: 820–831. doi: 10.1002/cncr.10215.
11. Aman NA, Doukoure B, Koffi KD, Koui BS, Traore ZC, Kouyate M, Toure I, Effi AB (2019) Immunohistochemical evaluation of Ki-67 and comparison with clinicopathologic factors in breast carcinomas. Asian Pac J Cancer Prev 20: 73–79. doi: 10.31557/APJCP.2019.20.1.73.
12. Leite M, Marques MS, Melo J, Pinto MT, Cavadas B, Aroso M, Gomez-Lazaro M, Seruca R, Figueiredo C (2020) *Helicobacter pylori* targets the EPHA2 receptor tyrosine kinase in gastric cells modulating key cellular functions. Cells 9: 627. doi: 10.3390/cells9020513.
13. Morgan E, Arnold M, Camargo MC, Gini A, Kunzmann AT, Matsuda T, Meheus F, Verhoeven RHA, Vignat J, Laversanne M, Ferlay J, Soerjomataram I (2022) The current and future incidence and mortality of gastric cancer in 185 countries, 2020-40: a population-based modelling study. EClinicalMedicine. 47: 101404. doi: 10.1016/j.eclinm.2022.101404.
14. Díaz P, Valenzuela Valderrama M, Bravo J, Quest AFG (2018) *Helicobacter pylori* and gastric cancer: adaptive cellular mechanisms involved in disease progression. Front Microbiol 9: 5. doi: 10.3389/fmicb.2018.00005.
15. Doorakkers E, Lagergren J, Engstrand L, Brusselaers N (2018) *Helicobacter pylori* eradication treatment and the risk of gastric adenocarcinoma in a Western population. Gut 67: 2092–2096. doi: 10.1136/gutjnl-2017-315363.
16. Tiwari SK, Shaik AS, Shaik AP, Alyousef AA, Bardia A, Habeeb MA, Khan AA (2019) Gene expression patterns of *COX-1*, *COX-2* and *iNOS* in *H. pylori* infected histopathological conditions. Microb Pathog 135: 103634. doi: 10.1016/j.micpath.2019.103634.
17. Cohen MC, Cueto Rúa E, Balcarce N, Quijano G, Drut R (2001) Focal atrophy with stellate scars in children with chronic gastritis associated to *Helicobacter pylori*. Is this early atrophic gastritis? Acta Gastroenterol Latinoam 31: 411–416. [Article in Spanish]. doi: 10.1002/j.1536-4801.2000.tb02816.x.
18. Cohen M, Cueto Rúa E, Balcarce N, Drut R (2004) Expression of cytokeratins 7 and 20 in *Helicobacter pylori*-associated chronic gastritis in children. Pediatr Dev Pathol 7: 180–186. doi: 10.1007/s10024-003-1006-4.
19. Filipe MI, Muñoz N, Matko I, Kato I, Pompe-Kirn V, Jutersek A, Teuchmann S, Benz M, Prijon T (1994) Intestinal metaplasia types and the risk of gastric cancer: a cohort study in Solvenia. Int J Cancer 57: 24-29. doi: 10.1002/ijc.2910570306.
20. Kirchner T, Müller S, Hattori T, Mukaisyo K, Papadopoulos T, Brabletz T, Jung A (2001) Metaplasia, intraepithelial neoplasia and early cancer of the stomach are related to dedifferentiated epithelial cells defined by cytokeratin-7 expression in gastritis. Virchows Arch 439: 512–522. doi: 10.1007/s004280100477.

21. Chen LW, Chang LC, Hua CC, Hsieh BJ, Chen SW, Chien RN (2017) Analyzing the influence of gastric intestinal metaplasia on gastric ulcer healing in *Helicobacter pylori*-infected patients without atrophic gastritis. BMC Gastroenterol 17: 1. doi: 10.1186/s12876-016-0563-8.

22. Jeong S, Choi E, Petersen CP, Roland JT, Federico A, Ippolito R, D'Armiento FP, Nardone G, Nagano O, Saya H, Romano M, Goldenring JR (2017) Distinct metaplastic and inflammatory phenotypes in autoimmune and adenocarcinoma-associated chronic atrophic gastritis. United European Gastroenterol J 5: 37–44. doi: 10.1177/2050640616644142.

23. Verheijen R, Kuijpers HJ, van Driel R, Beck JL, van Dierendonck JH, Brakenhoff GJ, Ramaekers FC (1989) Ki-67 detects a nuclear matrix-associated proliferation-related antigen. II. Localization in mitotic cells and association with chromosomes. J Cell Sci 4: 531–540. doi: 10.1242/jcs.92.4.531.

24. Nogueira Tde B, Artigiani RN, Herani BF, Waisberg J (2016) *H. pylori* infection, endoscopic, histological aspects and cell proliferation in the gastric mucosa of patients submitted to Roux-en-Y gastric bypass with contention ring: a cross sectional endoscopic and immunohistochemical study. Arq Gastroenterol 53: 55–60. doi: 10.1590/S0004-28032016000100011.

25. White NM, Gabril M, Ejeckam G, Mathews M, Fardy J, Kamel F, Doré J, Yousef GM (2008) Barrett's esophagus and cardiac intestinal metaplasia: two conditions within the same spectrum. Can J Gastroenterol 22: 369–375. doi: 10.1155/2008/243254.

26. O'Brien VP, Koehne A, Dubrulle J, Rodriguez AE, Leverich CK, Kong P, Campbell JS, Pierce RH, Goldenring JR, Choi E, Salama NR (2020) *Helicobacter pylori* accelerates KRAS-dependent gastric dysplasia. Life Sci Alliance 4: e202000967. doi: 10.26508/lsa.202000967.

Corresponding author

Aram Ommar, BSc, MSc.
Department of Clinical Pharmacy, College of Pharmacy, Hawler Medical University
100m Road, Erbil, Iraq
Tel: +964750 900 5010
Email: aram.ommar@hmu.edu.krd

Conflict of interests: No conflict of interests is declared.