Helicobacter Pylori as a Promoter of Accelerated Regeneration, Pathological Differentiation and Transformation of Normal Gastric Mucosa into Cancerous Type

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Abstract

The aim of the study was to ascertain presence of Helicobacter pylori in gastric carcinoma as a responsible promoter of inflammatory-regenerative changes, which lead to pathological differentiation and transformation of normal epithelial cells into intestinal type and, in progression, cause epithelial dysplasia that develops into early gastric carcinoma. The paper presents prospective study that includes clinical, pathohistological and microbiological aspects of carcinogenesis initiation in gastric mucosa. The subjects are patients treated at Gastroenterohepatology Clinic divided into two groups. One group included 50 patients with gastric carcinoma while the control group included 50 patients with chronic atrophic *H. pylori* positive gastritis. All the patients were subjected to endoscopy as well as biopsy targeted at antrum, lesser curvature and corpus and at the region 1-2 cm removed from tumor lesion. We used HUT test to verify *H. pylori* presence in biopsy samples. We analyzed the samples for presence, frequency and severity of inflammatory-regenerative, metaplastic and dysplastic changes in gastric mucosa and evaluated their meaning for the prognosis. Our study confirmed Helicobaster pylori responsibility for inflammatory events in gastric mucosa in patients with gastric carcinoma. Slight and mild epithelial dysplasia with chronic atrophic gastritis grade I and II coupled with intestinal metaplasia may be considered an indicator for early detection of carcinoma. Such patients represent risk group for gastric carcinoma development.

KEY WORDS: *Helicobacter pylori (H. pylori)*, intestinal metaplasia (IM), epithelial dysplasia, chronic atrophic gastritis

Introduction

Atrophic foci in gastric mucosa with altered mucus secretion are more vulnerable and susceptible to acid stomach content. Gastric mucosa is replaced by intestinal type due to aberrant differentiation. Detrimental factors further penetrate epithelia and, as a consequence, intestinal metaplasia develops into epithelial dysplasia (1,2,3). Pathohistologically, epithelial dysplasia is precursory lesion for multilayered, columnar epithelium carcinoma. Structural and cellular irregularities with preserved basal membrane are evident in this type of lesion. It represents focal proliferation of epithelial cells that is temporally unlimited. Individual cells with morphological characteristics of malignant cells occur due to the irregular differentiation – cellular atypia (4,5,6). In the appearance and function, thus altered epithelium does not represent clear malignant change. However, if the change tends to progress it may increase the risk of carcinoma development. Dysplasia may be reversible; it can improve if the outside factors that caused it are removed (7,8). According to the cancerous lesions classification proposed by the World Health Organization in 1978, epithelial dysplasia is classified as slight, mild (bordering) and severe dysplasia. Slight dysplasia occurs during regenerative changes in the cases of profound erosions as well as in hyperplastic lesions with slightly degraded structure. This type of epithelial dysplasia may still revert to normal mucosa. Mild dysplasia (bordering lesion) represents marked disorder of gland structure, expansion of mitotic zone and atypical epithelium with characteristics that suggest its progression towards severe dysplasia or "carcinoma in situ". Severe epithelial dysplasia is related to "carcinoma in situ" and adenocarcinoma lacking invasive growth (9,10,11). In praxis, it is difficult to differentiate "carcinoma in situ" from this type of dysplasia due to inconsistencies in the degree of cellular atypia and structural disorder (12,13,14,15,16). .

Material and Methods

The study is of prospective character and includes 50 patients with gastric carcinoma and 50 patients with chronic atrophic *Helicobacter pylori (H. pylori)* positive gastritis that are treated at Gastroenterohepatology Clinic, Sarajevo University Clinics Center. All the patients were subjected to endoscopy and biopsy targeted at antrum, lesser curvature, corpus and the area surrounding carcinoma at the distance of 1-2 cm. All the carcinoma cases were pathohistologically verified. Mucosa specimens collected by biopsy were conserved in 10% neutral buffered formalin, paraffin embedded and sliced with microtome into 5 μm thick sections. Tissue was stained with Haematoxylin and Eosin (HE staining). The presence and level of inflammatory-regenerative changes was examined in all the patients. We graded inflammatory-regenerative changes as chronic superficial, chronic atrophic gastritis grade I, II or III based on the defined histological criteria. The level of activity, defined as active phase or dormant phase, was determined based on the presence of leukocyte infiltration. Based on the defined histological criteria for easy differentiation between inflammatory-regenerative and dysplastic changes, epithelial dysplasia was classified as slight, mild or severe dysplasia. Differentiation was done after the analysis of 19 criteria, which were visually graded 1-4 based on the intensity. The examined criteria included: the size and the shape of nuclei, nuclei chromacity, chromatin distribution within nuclei, prominence and number of nucleoli, nucleus/cytoplasm ratio, nuclei distribution, cytoplasm staining, integrity of cell types within glands, quantity of the secreted mucus, number of mucous glands, appearance of budding or branching, villous appearance of mucosa surface, occurrence of "back to back" formations, presence or absence of cellular infiltration in lamina propria and occurrence of abscesses in crypts. Following the change assessment as inflammatory-regenerative or dysplastic, the tissue was prepared for immunohistochemical analysis. It was HE stained earlier. Intestinal metaplasia was included into one of the three available categories based on the detailed mucine analysis and morphological changes. We used fast Helicobacter Urease Test (HUT) - Astra, for verification of Helicobacter pylori presence in biopsy samples collected from antrum and corpus. Control group was represented by patients with *H. pylori* positive gastritis. Based on macroscopically observed changes, we endoscopically biopsied mucosa in antrum, lesser curvature and corpus. Biopsy specimens were prepared in the same manner as in the other group of patients. Based on the established criteria, we determined and graded inflammatory-regenerative, dysplastic changes in gastric mucosa and immunohistochemically typed intestinal metaplasia. .

Results

The first group of subjects included patients with gastric carcinoma. There were 28 male patients 58 years old on average and 22 female patients 62 years of average age. We classified carcinoma cases according to regions based on previously established criteria. Most of the cancerous formations were located in antro-pyloric region (66%). Epithelial dysplasia distribution according to regions is presented in tables.

	SUPERFICIAL	CAG gr. I	CAG gr. II	CAG gr. III
Slight dysplasia	0	21 (80,77%)	5 (19,23%)	0
Active phase	0	20 (76,92%)	5 (19,23%)	0
Dormant phase	0	1 (3,85%)	0	0

TABLE 1. Epithelial dysplasia distribution according to chronic gastritis grade and the activity in the group of patients with slight dysplasia - antral region

	SUPERFICIAL	CAG gr. I	CAG gr. II	CAG gr. III
Mild dysplasia	0	3 (50%)	3 (50%)	0
Active phase	0	3 (50%)	3 (50%)	0
Dormant phase	0	0	0	0

TABLE 2. Epithelial dysplasia distribution according to chronic gastritis grade and the activity in the group of patients with mild dysplasia - antral region

	SUPERFICIAL	CAG gr. I	CAG gr. II	CAG gr. III
Slight dysplasia	0	13 (72,22%)	5 (27,78%)	0
Active phase	0	13 (72,22%)	5 (27,78%)	0
Dormant phase	0	0	0	0

TABLE 3. Epithelial dysplasia distribution according to chronic gastritis grade and the activity in the group of patients with slight dysplasia – area around carcinoma

	SUPERFICIAL	CAG gr. I	CAG gr. II	CAG gr. III
Mild dysplasia	0	6 (66,67%)	3 (33,33%)	0
Active phase	0	6 (66,67%)	3 (33,33%)	0
Dormant phase	0	0	0	0

TABLE 4. Epithelial dysplasia distribution according to chronic gastritis grade and the activity in the group of patients with mild dysplasia – area around carcinoma

	SUPERFICIAL	CAG gr. I	CAG gr. II	CAG gr. III
Slight dysplasia	5 (10%)	4 (8%)	2 (4%)	0
Active phase	4 (8%)	4 (8%)	2 (4%)	0
Dormant phase	1 (2%)	0	0	0

TABLE 5. Distribution of slight epithelial dysplasia according to chronic gastritis grade – corpus

Analyzing epithelial dysplasia in the control group we found the results presented in the following tables.

	SUPERFICIAL	CAG gr. I	CAG gr. II	CAG gr. III
Slight dysplasia	0	19 (70,37%)	8 (29,63%)	0
Active phase	0	19 (70,37%)	8 (29,63%)	0
Dormant phase	0	0	0	0

TABLE 6. Distribution of slight epithelial dysplasia in the control group – antrum

	SUPERFICIAL	CAG gr. I	CAG gr. II	CAG gr. III
Slight dysplasia	3 (6%)	6 (12%)	6 (12%)	0
Active phase	2 (4%)	5 (10%)	6 (12%)	0
Dormant phase	1 (2%)	1 (2%)	0	0

TABLE 7. Distribution of slight epithelial dysplasia in the control group – lesser curvature

Discussion

In our study of the distribution of epithelial dysplasia in various stomach regions, we found slight epithelial dysplasia in antral region in 26 of 50 (52%) patients with gastric carcinoma. In 80,77 % patients dysplasia was coupled with chronic gastritis grade I (CAG I), which was active in 76,92 %. Chronic gastritis grade II in active phase was identified in 19,23% cases. Mild epithelial dysplasia was identified in 6 (12%) cases. Of those, 3 (50%) had CAG grade I and 3 (50%) had CAG grade II in active phase. Intestinal metaplasia was identified in 32 subjects (64%). Rank correlation (Wilcox test) between epithelial dysplasia and intestinal metaplasia in antral region was sig-

nificantly high (significance threshold p=0, 05). 18 (36%) subjects were found to have slight epithelial dysplasia in the area around carcinoma. CAG grade I in active phase was associated with 72,22% slight dysplasia cases. In 27,28% cases, dysplasia was coupled with CAG grade II in active phase. Mild dysplasia was established in 9 (18%) subjects. 66,67 % had CAG grade I in active phase while 33,33% had CAG grade II in active phase. Cellular atypia was characteristic for the area around carcinoma. Our findings confirmed 22% subjects with cellular atypia also had CAG grade I while 6% had CAG grade II. Gastritis was in active phase. We were unable to identify gastritis grade in one subject. In corpus region slight epithelial dysplasia was differentiated in 11 (22%) patients. Superficial gastritis was found in 10%, CAG grade I in 8% and CAG grade II in 4% patients. According to Wilcox rank correlation test significant correlation was established between gastritis type and the presence of slight dysplasia in the area of corpus in patients with gastric carcinoma (p=0,0069, d=14, significance threshold p=0,01). Mild dysplasia was not found. In control group, we found slight dysplasia in antral region in 27 of 50 (54%) subjects; 70,37% had CAG grade I in active phase while 29,63 had CAG grade II in active phase. Mild dysplasia was identified in 5 (10%) subjects. 80% of those had CAG grade I in active phase while 20 % had CAG grade II, also active. In lesser curvature area epithelial dysplasia was identified in 15 (30%) subjects. In 20% cases it was associated with superficial while in 50% patients it was coupled with CAG grade I and CAG grade II. Only 6,64% cases of gastritis were in dormant phase. Mild dysplasia was identified in 4 (8%) patients. It was associated with CAG grade I in 3 (75%) cases and CAG grade II in one case of four. All those gastritis cases were in active phase. Three (6%) slight dysplasia cases were found in the corpus region. All of those were associated with CAG grade II in active phase. Mild and severe cases of epithelial dysplasia were not identified. Examining the area around cancerous growth no significant correlation between epithelial dysplasia and intestinal metaplasia was found. However, in the region of corpus correlation is significant p=0,0015 for significance threshold p=0,005. Presence of *Helicobacter pylori* in the mucosa initiates inflammatory process and is responsible for superficial gastritis, which progresses into severe grades coupled with the loss of epithelial cells and the atrophy of gastric glands.

Conclusion

Comparing our results against published studies we reached almost identical conclusions. Our study confirmed chronic atrophic gastritis in active phase coupled with the progression of epithelial dysplasia. The progression was particularly evident in the area around carcinoma with dominant cellular atypia as a sign of the progression of slight into mild and bordering epithelial dysplasia, which was related to the presence of cancerous growth in gastric mucosa. We have proven high correlation between epithelial dysplasia and chronic gastritis in antral region as well as between intestinal metaplasia and epithelial dysplasia. In our patients group, cancerous growth was most frequently located in antral region of the stomach.

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