Ultrastructural study of the gastric mucosa and helicobacter pylori in duodenal ulcer patients

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ABSTRACT

Objectives: To investigate the relationship between Helicobacter pylori and gastric mucosa in control and duodenal ulcer patients at the electron microscopic level.

Methods: Three antral biopsies were taken from each of 20 normal control volunteers and 30 duodenal ulcer patients presented to the gastroenterology unit at Jordan University Hospital for upper endoscopic examination. Each specimen was fixed and processed for electron microscopic study.

Results: Two types of Helicobacter pylori were observed and identified by their morphology at electron microscopy. The first one was characterized by double external smooth membranes and homogeneous cytoplasmic contents, and the second type with a characteristic ring-shaped intracytoplasmic vacuole. Electron microscopic examination of normal controls showed normal gastric mucosa and a small number of Helicobacter pylori in 12 out of 20 controls. However, in duodenal ulcer patients, 5 different patterns of interaction between the Helicobacter pylori and gastric mucosa were observed in relation to the severity of the disease. In duodenal ulcer patients, various types of epithelial damage was seen accompanied with a decrease or absence of mucous secretion and with more colonization of bacteria.

Conclusion: The morphology and pathogenesis of Helicobacter pylori was described in duodenal ulcer patients, and 5 different patterns of contact between Helicobacter pylori and surface epithelium were recognized causing variable degrees of microvillous atrophy and reduced mucous secretion. The vacuolated type of Helicobacter pylori was more adherent to the damaged epithelium and there was a direct relationship between the epithelial damage and bacterial load. In the normal controls, no epithelial damage and scanty bacteria were observed. The various types of epithelial changes of gastric mucosa has initiated more research at electron microscopic level on the immune mechanism of the gastric mucosa to determine the underlying cause of the varying severity of the disease.

Keywords: Ultrastructure, gastric mucosa, Helicobacter pylori, duodenal ulcer.

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Several morphological studies have been reported regarding the forms and interactions of Helicobacter pylori (H.pylori) with gastric mucosa,[1-3] but little is known about the exact mechanisms of the bacterial pathogenicity. H.pylori is considered a major factor in the pathogenesis of peptic ulcer diseases,[4,5] chronic gastritis[6,7] and perhaps gastric malignancy.[8] Detection of H.pylori is usually performed by culture, polymerase chain reaction, histology or urease test on gastric biopsy samples.[9] Recently, a large number of studies have demonstrated that H.pylori vacuolating cytotoxin (CagA) may be involved in the pathogenesis of the bacterial infection,[10] and is highly associated with

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different gastroduodenal diseases.[11-15] H.pylori is found in more than 90% of duodenal ulcer patients. Its exact pathogenic role has not been elucidated. Treatment options have focused on the eradication of the organism, and a wide range of results have been reported[16] and the matter continues to be vigorously debated.

The aim of this study is to investigate the possible relationship between H.pylori and gastric mucosa in normal subjects and in duodenal ulcer patients at the electron microscopic level. It is hoped that such ultrastructural studies would lead to a better understanding of the pathogenetic mechanisms between gastric epithelium and H.pylori.

Methods. Twenty normal asymptomatic control volunteers, age and sex matched with 30 duodenal ulcer patients were enrolled in this study. Informed consent was obtained from all patients. Three biopsies of gastric antrum were taken from each normal control and duodenal ulcer patient presented to the gastroenterology unit at Jordan University Hospital for upper endoscopic examination. Biopsies from the normal volunteers were included in the study for comparison, at the electron microscopic level, with those of patient specimens. One specimen was taken from the lesser curvature aspect of the antrum while the remaining 2 specimens were obtained from the opposite site from the greater curvature of the stomach. Each specimen was cut into 3 small pieces and fixed in a solution of 1.25% glutaraldehyde and 1% paraformaldehyde in O.I M phosphate buffer (pH 7.2-7.4) for 48 hours at 4°C. All specimens were postfixed in 1% osmium tetroxide in the same buffer at 4°C for 2 hours. The tissues were dehydrated in graded ethanol and then embedded in araldite. Semi-thin 1µm sections were cut, stained by toluidine blue and were examined under light microscopy to localize the H.pylori in relation to the gastric epithelium. Ultra-thin sections of the selected areas were then cut and stained with 3% uranyl acetate and lead citrate and examined with the Philips 200 transmission electron microscope. The disease activity was assessed according to bacterial load, quality of mucous, microvillus atrophy and vacuolation of the lining epithelium. It was classified as mild, moderate or severe activity as shown in Table 1.

Results. H.pylori was seen in all specimens of duodenal ulcer patients and in 60% of controls. The bacteria was helical-shaped, with a unipolar sheathed flagella, and a size of 3-5µm in length and 0.3 - 0.8 µm in diameter (Figure 1). Two forms of H.pylori were identified at the electron microscopic level; one with external smooth double membranes and homogeneous cytoplasmic contents (Figure 1). Its external membrane was denser, ring-like and

Table 1 - The criteria used in the classification of the severity of the disease.

	Mild	Moderate	Severe
Mucous	Normal	Vacuolation	Absent
Microvillus atrophy	Partial	Subtotal	Total
Vacuolation of lining epithelium	None	Mild	Severe
Number of bacteria	No/Scanty	Moderate	Abundant

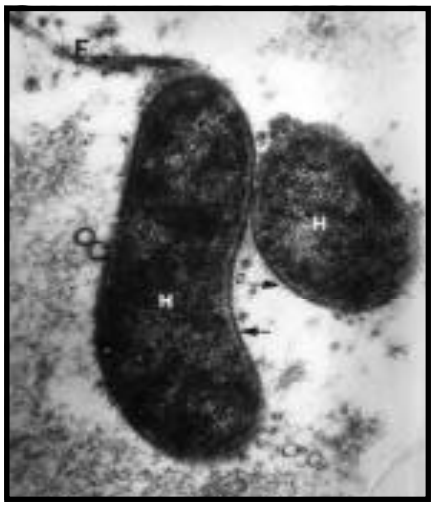

Figure 1 - Electron micrograph of gastric biopsy specimen showing the shape of H.pylori with external smooth double membranes (arrows) and homogeneous cytoplasmic contents (H), sheathed flagella (F), and circular parts of the flagella observed near the organism. (Magnification x 28,000).

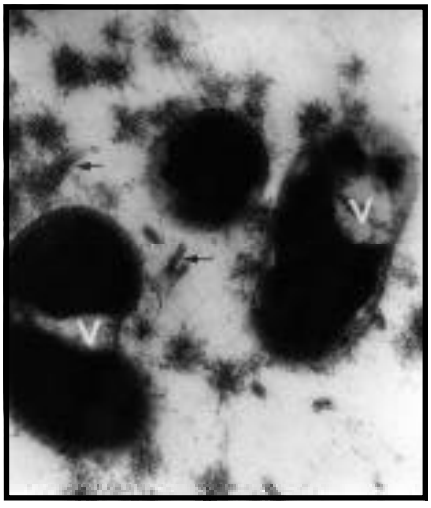

Figure 2 - Electron micrograph of gastric biopsy specimen showing H.pylori with ring shaped intra-cytoplasmic vacuoles (V), parts of the sheathed flagella observed near the organism (arrows). (Magnification x 45,000).

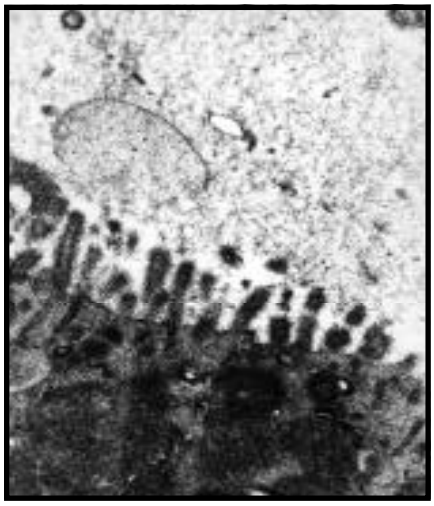

Figure 3 - Electron micrograph of control gastric biopsy specimen showing normal gastric mucosa without bacteria. (Magnification x 17,000).

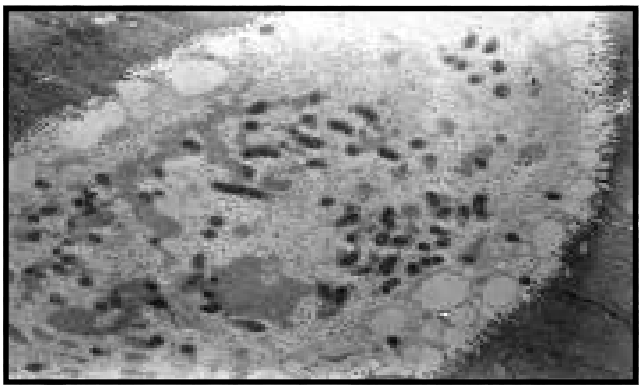

Figure 4 - Electron micrograph of gastric biopsy specimen of duodenal ulcer patient showing H.pylori in the mucous secretion away from a healthy gastric mucosa. (Magnification x 3800).

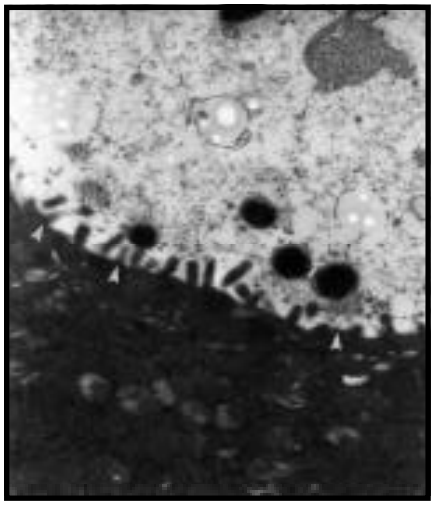

Figure 5 - Electron micrograph of gastric biopsy specimen of duodenal ulcer patient showing H.pylori close to the microvilli with less mucous secretion and with slight damage (arrow-heads) to the microvilli of the glands. (Magnification x 10000).

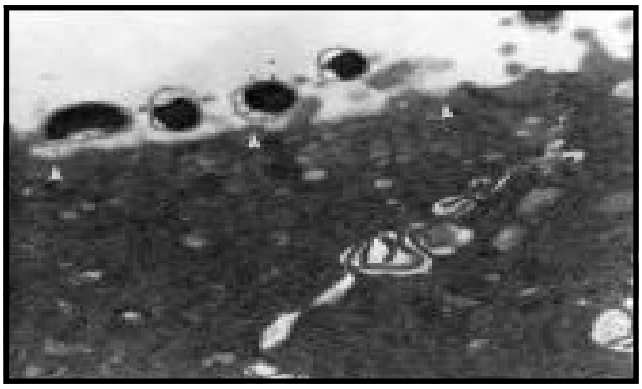

Figure 6 - Electron micrograph of control gastric biopsy specimen of duodenal ulcer patient showing H.pylori resting on the epithelial tissue without mucous secretion and with few or loss of the microvilli (arrow-heads). (Magnification x 6300).

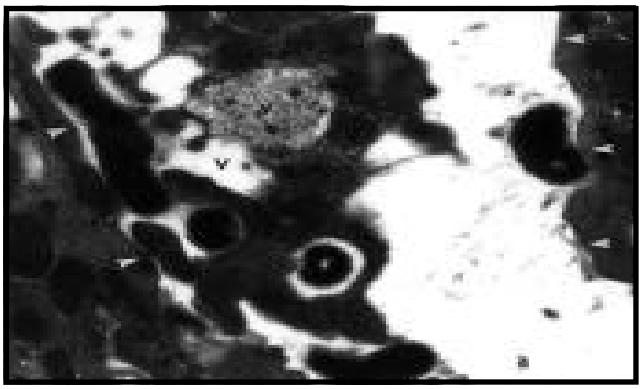

Figure 7 - Electron micrographs of gastric biopsy specimen of duodenal ulcer patient showing H.pylori without mucous secretion and with severe damage of epithelial tissues, loss of the microvilli (arrow-heads) and vacuolated epithelial gland (v) is observed. (Magnification x 17000).

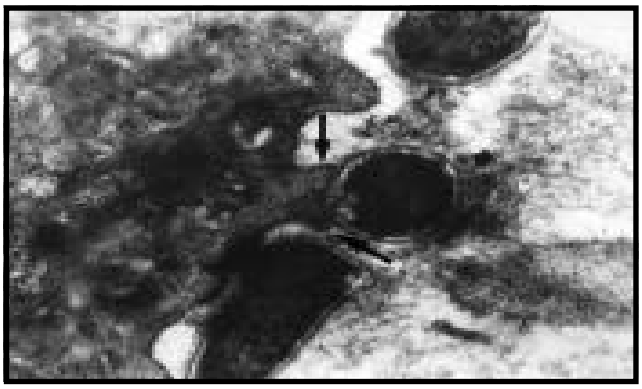

Figure 8 - Electron micrograph of gastric biopsy specimen of duodenal ulcer patient showing H.pylori adherent to the epithelial tissue with specific junction zones (arrows). (Magnification x 35000).

measured 12-15µm in diameter. Numerous circular parts measuring 30µm in thickness were observed near one pole of the organism representing extension of the flagellar sheath (Figures 1 & 2). The second form predominantly contained ring-shaped intra-cytoplasmic vacuoles (Figure 2), which were of variable sizes. Electron microscopic examination of gastric mucosa in normal subjects revealed that H.pylori was seen in 12 cases out of the 20 with normal epithelial tissue (Figure 3). In the duodenal ulcer patients, on the other hand, 5 different phases of interaction between the H.pylori and gastric mucosa were observed and classified into: Phase 1: H.pylori seen free in the mucous secretion and away from the healthy microvilli of an intact gastric epithelium (Figure 4). Phase 2: H.pylori located close to the microvilli of the epithelial glands with decreased mucous secretion and partial microvillous atrophy (Figure 5). Phase 3: H.pylori resting on the epithelial tissue without mucous secretion and subtotal villous atrophy (Figure 6). Phase 4: H.pylori seen very close to the damaged epithelium with absent mucous secretion. Severe damage of epithelial tissue in the form of total microvillous atrophy and vacuolated epithelial glands was observed (Figure 7). Phase 5: H.pylori adherent to the epithelial tissue with specific junction zones, which appeared as direct contact without membrane separation in the absence of mucous secretion (Figure 8). In all specimens of duodenal ulcer patients, the gastric-lining epithelium in areas colonized by bacteria showed different patterns of morphological changes, such as, variable degrees of microvillus atrophy (Figures 6 & 7), and vacuolation of the epithelial glands (Figure 7). However, no morphological changes were ever observed in any of the control cases (Figure 3), inspite of the presence of H.pylori in 60% of cases.

Discussion. Several morphological studies at electron microscopic level have contributed important information on the relationship between H.pylori and gastric mucosa.[1-3,6] In this study, the duodenal ulcer patients exhibited 2 forms of H.pylori; one with a homogeneous cytoplasm and double external membrane, while the second persistently showed an intracytoplasmic ring-shaped vacuole, which was always in direct contact with the damaged epithelium. The patterns of contact between the H.pylori and epithelial tissue were studied and classified into 5 different phases related to the variety of pathological changes ranging from reduced mucous secretion to total microvillus atrophy. Caselli et al[1] studied the ultrastructural patterns of H.pylori and concluded that the 2 forms of bacterial patterns do exist, and the second one seemed to be the only form related to the damaged epithelium. He also reported that the intrabacterial vacuoles of the second type of H.pylori contain polyphosphates, which are energy reservoirs used in the absence of exogenous energy. The intracytoplasmic vacuoles usually arise in areas where degenerated epithelium exist.[1,17] Recently Janas et al[2] studied the relationship between the helical and coccoid forms of H.pylori and the gastric epithelial cells in positive patients. He concluded that the coccoid forms are closely associated with damaged gastric mucous cells. Two types of coccoidal forms have been described commonly in cultures.[18] They emerge in culture, and under unfavorable conditions they are inactive or dormant. In our study we did not come across a significant number of coccoid forms of H.pylori, which can be explained by the possibility that such forms exist most commonly in patients with gastric adenocarcinoma. These coccoidal forms were more frequently found in cases of adenocarcinoma than in cases of benign ulcers.[18]

In this study, the epithelial changes observed in the duodenal ulcer patients and the patterns of contact between the bacteria and gastric epithelium were described and classified into 5 different phases in relation to the severity of the diseases. We noted that one or more patterns of interaction between H.pylori and the gastric epithelium could be observed in a particular ulcer patient. The organism was usually seen on the surface epithelium and within the gastric pits, but intracellular invasion was never observed. This could be attributed to the low virulence of H.pylori.[5,19] In severe epithelial damage, a relationship between the mucous secretion and various types of epithelial changes including; destruction of gastric cell membrane, vacuolation of the epithelial glands, distortion or atrophy of the microvilli was observed. The direct contact between H.pylori and surface epithelium seems to be a vital step prior to significant mucosal damage. It is well known that the H.pylori is able to produce large quantities of urease enzyme which converts urea present in the gastric juice into bicarbonate and ammonia. This provides an alkaline media that causes damage to the gastric mucosa. Once the H.pylori take residence in the mucous, it produces more urease enzyme, and thus colonization occurs.[17,19]

The Junction zone patterns observed in our study are really unique. The explanation for this phenomena is not yet well understood. However these junction zones may represent areas of pseudo-polyp formation by the denuded mucosa on either side of the zone resulting from extensively damaged epithelium. The decreased mucosal integrity due to severe infection causes elaboration of bacterial toxins and induction of tissue injury. However, ultrastructural studies confirm the mucous depletion and changes in surface epithelium.[1-3,5] Since, the control cases in our present study showed a smaller number of bacteria with normal gastric mucosa, therefore the role of the gastric immune system in

modulating H.pylori infection requires further study. The results of this study which describe the morphology and pathogenesis of H.pylori in duodenal ulcer patients and the epithelial changes of gastric mucosa at electron microscopic level will initiate more research on the immune mechanism of the gastric mucosa in order to determine the underlying cause of the varying severity of the disease.

The results of our study sheds more light on the natural history of H.pylori infection and the gastric epithelium which, we hope will enable clinicians and researchers to have a better understanding of peptic ulcer disease caused by this bacteria. These results may possibly help in better eradication regimens and future development of vaccines and prevention of gastric malignancy.

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