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Clinical Experience

# Ultrastructure Characteristics of Different Chinese Medicine Syndromes of *Helicobacter pylori*-Correlated Gastric Diseases*

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**ABSTRACT** **Objective:** To explore the ultrastructure characteristics of patients with dampness-heat of Pi (Spleen)-Wei (Stomach) syndrome (DHPW) and Pi-qi deficiency syndrome (PQD), both of which are *Helicobacter pylori* (*Hp*)-correlated gastric diseases (HPCG), and implicate a helpful hint for the clinical microcosmic syndrome differentiation. **Methods:** Fourteen gastric mucosa samples from 6 chronic gastritis (CG) and 6 active peptic ulcer (including 8 DHPW, 4 PQD) as well as 2 healthy volunteers were collected and tested for *Hp* infection. The ultrastructure of gastric mucosa was observed under the transmission electron microscope (TEM). **Results:** Among 14 gastric mucosa samples, 8 of them were *Hp* positive (6 DHPW and 2 PQD), which were all accordance with the results screened by supermicro-pathological method. Under TEM, the normal gastric mucosa, with tidy microvilli and abundant in mucus granules, mitochondria and rough endoplasmic reticulum distributed evenly, and with smooth nucleus membrane. But in those specimens of DHPW with *Hp* infection, microvilli were presented with burr shape. Especially, those samples from dampness-heat syndrome with predominant heat type (DHSH) patients were more obvious, with microvilli damaged, mitochondria concentrated and distributed in disorder, secretory tubule extended. In dampness-heat syndrome with predominant dampness type (DHSD) patients, mucus granules aggregated obviously, mitochondria swelled and blurred, and rough endoplasmic reticulum crowded. For 2 samples of DHPW without *Hp* infection, their microvilli were intact, with mitochondria increased and gathered but well-distributed, and secretory tubule extended mildly. In 2 PQD patients with *Hp* positive, the specimens of microvilli were sparse, and their mucus granules and mitochondria were decreased, with fractured crests and vacuole, secretory tubules extension to nucleus membrane, and rough endoplasmic reticulum extension in a pool-like way, and nucleus condensed. The 2 samples from PQD patients without *Hp* infection were characterized with intact microvilli, decreased mitochondria, fractured crest and extended rough endoplasmic reticulum in a pool-like way. **Conclusion:** It is obvious different in ultrastructure of DHPW and PQD patients under TEM, which may give a helpful hint for the microcosmic syndrome differentiation of HPCG.

**KEYWORDS** *Helicobacter pylori*-correlated gastric diseases, supremicro-pathology, dampness-heat syndrome of Pi (Spleen) and Wei (Stomach), Pi-qi deficiency, Chinese medicine

*Helicobacter pylori* (*Hp*), colonized in the stomach of more than 50% of people worldwide, is one of the crucial factors that attacks human gastric mucosa, with explicit correlation of chronic gastritis, peptic ulcer (PU), precancerous lesions and even gastric cancer.[1] With increasing in *Hp* infection rate and drug resistance, it's urgent to combination Chinese medicine (CM) with Western medicine to relieve the patients' symptoms, increase therapeutic effects and reduce toxin. This study was designed to observe the difference of supermicro-structure of gastric mucosa in patients with *Hp*-correlated gastric diseases (HPCG) and explore the method of syndrome differentiation for CM in a microcosmic way.


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## METHODS

### Diagnostic, Inclusion and Exclusion Criteria

The diagnosis of chronic gastritis (CG) and PU were referenced on the diagnostic standards on CG,[2,3] and the diagnosis of *Hp* infection was accordance with the standards of The third report of Chinese *Helibaceter pylori* infection.[4] Furthermore, the diagnosis of dampness-heat of Pi (Spleen)-Wei (Stomach) syndrome (DHPW) was established based on previous study by our team,[5] and the diagnosis of Pi-qi deficiency syndrome (PQD) was based on the National Administration of Traditional Chinese Medicine in 2002.[6] All the included subjects of CG or PU (including CM syndrome of DHPW or PQD) were aged 18–80 year-old, and each patient signed informed consent. People who were tested negative in regular physical examination with normal tongue and pulse manifestation were chosen as healthy volunteers. Patients who had used antibiotics and anti-acid drugs 1 month before or suffered from heart, liver, kidney, lung or other system disease; those who were pregnant or in lactation period; those who obviously lost weight, with melena and (or) were diagnosed with gastric cancer by gastroscope, were excluded.[5]

From March 2009 to February 2010, 14 gastric samples from 6 CG, 6 active PU and 2 healthy volunteers were collected from clinic of the First Affiliated Hospital of Guangzhou University of Chinese Medicine. All the included subjects were reported with questionnaire surveys by 2 experts with intermediate or senior title of CM to record the tongue picture and pulse manifestation. The study was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou University of Chinese Medicine (No. 2015-009).

### Specimen Collection and Preparation

After the patients underwent a gastronsopy and were diagnosed with CG or active PU, 2 specimens of gastric mucosa in the antrum with distance of 2–3 cm from pylori were collected. One specimen was tested with rapid urease test (RUT) immediately, the other was fixed with 3% glutaraldehyde (TED PELLA, INC, USA), dehydrated, and embedded in order to observe the transformation of substructure and *Hp* infection.

### Uranyl Acetate and Lead Stain

The fresh gastric mucosa was fixed with 3% glutaraldehyde (4 ℃, 3 h), and washed with phosphate-buffer saline (PBS, 0.1 mol/L, 10 min × 2). Subsequently, the gastric mucosa was osmic acid re-fixed (1%, 2 h), and washed with PBS; following dehydrated with ethanol (50%, 10 min; 70%, 10 min), re-dehydrated with different concentration (80%, 90%, and 100%, respectively) of acetone (10 min, twice) at each concentration. After exchanging the acetone of embedding medium with Epon812 (1:1) for 40 min, the new embedding medium were saturate overnight (37 ℃) and the dehydrated gastric mucosa was put into the saturated Epon812 embedding medium, embedded and aggregated (60 ℃, 48 h).

Under anatomic microscope, the gastric mucosa exposed from the embedding were fixed as a trapezoid-shaped. Then the specimen was sliced into 1 μm thin sheet on an ultrathin slicing machine (AO, Austria) and flattened. After stained with hematoxylin-eosin (HE), orientation under the anatomic and general microscope. Subsequently, the oriented section (0.09 $mm^2$) was sliced into 40–60 nm, and was removed the super-slice with copper net. Then stained with saturated uranyl acetate prepared with 70% ethanol for 3 min, and washed with double-distilled water for 5 min and dried. Finally, the supermicro morphology of gastric mucosa was screened and *Hp* infection under the TEM was observed (Hitachi Asia Ltd, Japan).

### Statistical Analysis

All statistical analyses were performed using SPSS software version 22.0 for Windows. Data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Differences between groups were determined using the Student's *t*-test. A two-tailed value of $P<0.05$ was considered statistically significant.

## RESULTS

### Clinical Parameters

The patients of 2 CM syndromes type included 8 males and 4 females (average age: 35.17 ± 9.10 year-old), and the average clinical course were 2.40 ± 1.63 years. And the patients of PQD included 2 males and 2 females(average age: 39.25 ± 8.54 year-old), with average clinical course of 3.19 ± 2.41 years. Six patients who were DHPW infected with *Hp* include 2 dampness-heat syndrome with predominant dampness (DHSD), 2 dampness-heat syndrome with predominant heat (DHSH) and 2 dampness-heat combined sickness (DHCS). Additionally, 2 healthy volunteers (1 female and 1 male, average age 29.67 ± 6. 24 year-old) were included. The clinical parameters for all included

**Table 1. Clinical Parameters for Included Individuals**

| Group | Case | Gender | | Disease | |
|---|---|---|---|---|---|
| | | Male | Female | PU | CG |
| DHPW | 8 | 6 | 2 | 4 | 4 |
| *Hp*(+) | 6 | 5 | 1 | 4 | 2 |
| *Hp*(-) | 2 | 1 | 1 | 0 | 2 |
| PQD | 4 | 2 | 2 | 2 | 2 |
| *Hp*(+) | 2 | 1 | 1 | 1 | 1 |
| *Hp*(-) | 2 | 1 | 1 | 1 | 1 |
| HV | 2 | 1 | 1 | — | — |

Notes: HV: healthy volunteers; PU: peptic ulcer; CG: chronic gastritis

individuals are listed in Table 1.

## *Hp* Infection Detection

By RUT, the specimens test results showed that 8 patients with *Hp* infection included 6 DHPW and 2 PQD. Furthermore, screened by electron microscope, the results of UALS showed that all *Hp* positive specimens were screened with curved or spiral bacillus dwelled in the surface of epithelium or deep mucous layer (Figures 1A–C).

Normal gastric mucosa showed tidy microvilli, enrich and well-distributed mucous granules (Figure 1D). The mitochondria of parietal cells were circled with nucleus, well-distributed, and normal structure, additionally, secretory canaliculus could be screened with secretory canaliculus and smooth nuclear membrane (Figure 1E). Endoplasmic reticulum and mitochondria were well-distributed on chief cells (Figure 1F).

## Subcellular Structure of HPCG Specimen with Different CM Syndrome

### Subcellular Structure of DHPW With or Without *Hp* Infection

For DHSH type specimen with *Hp* infection, epithelial microvilli were burr in shape, mucous granules aggregation in the internal of capsule and lysosome increased (Figure 2A). Parietal cells were vacuolus, a large number of mitochondria was present there, they were variable in shape and arrange (Figure 2B). The secretory tubules extended and distributed with cluster type (Figure 2C). Pyknosis nuclear membrane could be observed (Figure 2D). In addition, rough endoplasmic reticulum of chief cell increased, and with lobular pyknosis nuclear membrane (Figure 2E).

For DHSD type specimen infected with *Hp*, microvilli of epithelial cell was presented with burr shape where *Hp* cluster (Figures 3A and 3D), and a large number of mucus particles could be seen within the inner membrane. Some fractured and edema mitochondria crests with rough endoplasmic reticulum increased and pyknosis nuclear membrane rough endoplasmic reticulum were crowded (Figures 3B and C). Pyknosis nuclear membrane was accompanied with apoptotic body and many mucus granules in the margin of membrane, where *Hp* colonizes (Figure 3D).

For DHCS type specimen with *Hp* infection, edema epithelial cells were in irregular shape, mucus granules fall off, and *Hp* gathered in the deep layer of mucus (Figure 3E). The edema mitochondria

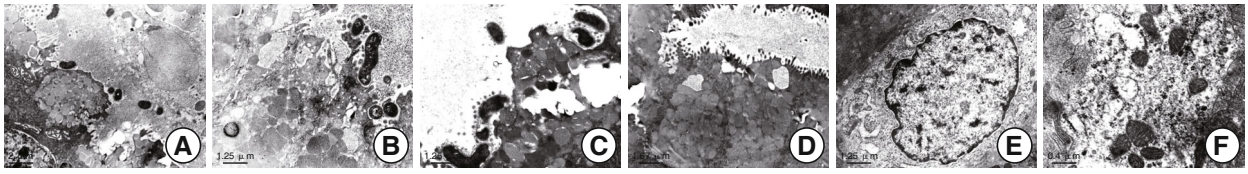


**Figure 1. *Helicobacter pylori* Colonization by UALS and Subcellular Structure of Normal Gastric Mucosa under Transmission Electronic Microscope**

Notes: A–C: *Hp* colonization (×5,000, 8,000); D: epithelial cell (×6,000); E: parietal cell (×8,000); F: chief cell (×25,000)

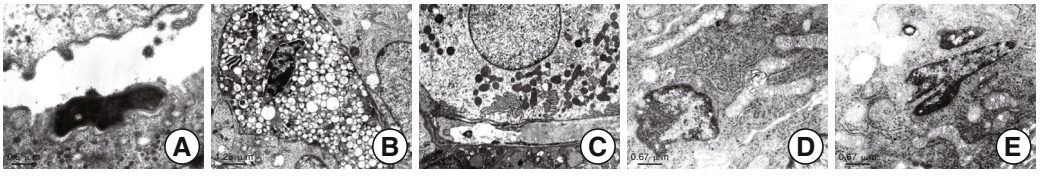


**Figure 2. Transformation Feature of Subcellular Structure of Dampness-Heat Syndrome with Predominant Heat with *Hp* Infection under TEM**

Notes: A: epithelial cell (×20,000); B-D: parietal cell (×6,000, 8,000, 15,000); E: chief cell (×15,000); TEM: transmission electronic microscope

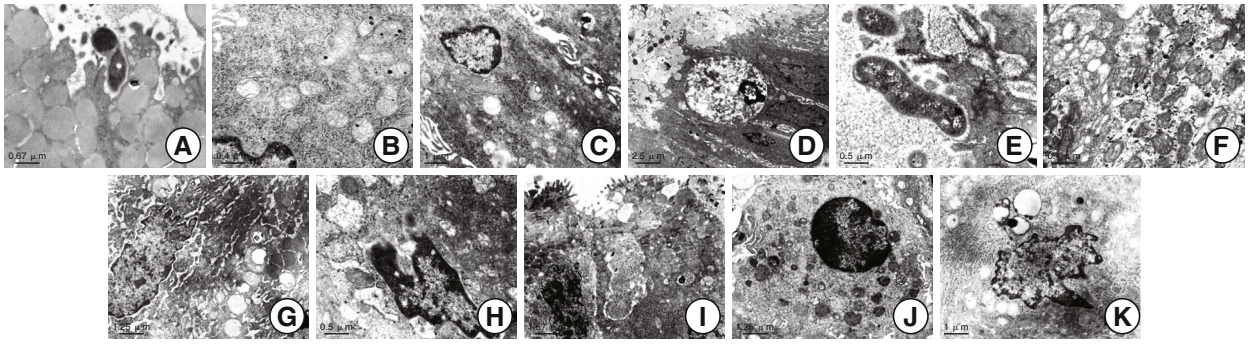


**Figure 3. Transformation Feature of Subcellular Structure of DHSD and DHCS Under TEM**

Notes: A–D: DHSD with *Hp* infection. A: epithelial cell (×15,000); B: parietal cell (×25,000); C, D: chief cell (×10,000, ×4,000). E–H: DHCS with *Hp* infection. E: epithelial cell (× 20,000); F: parietal cell (×25,000); G, H: chief cell (×8,000, ×20,000). I–K: DHPW without *Hp* infection. I: epithelial cell (×6,000); J: parietal cell (×8,000); K: chief cell (×10,000)

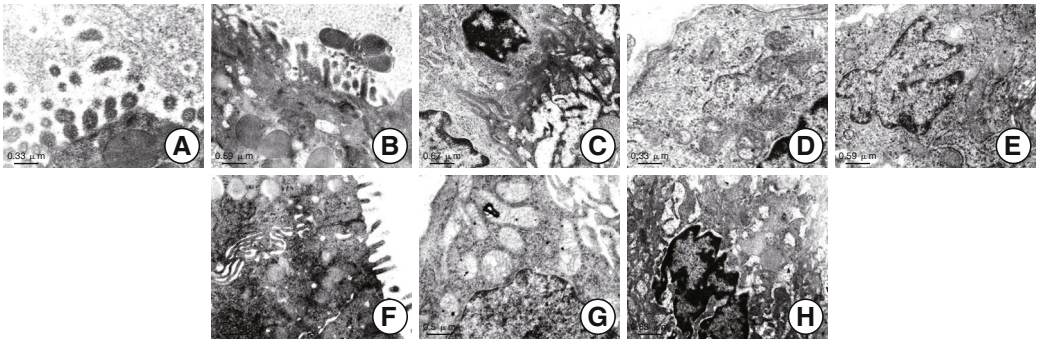


**Figure 4. Transformation Feature of Subcellular Structure of Pi-qi Deficiency Syndrome Specimen With or Without *Hp* Infection Under TEM**

Notes: A-E: PQD with *Hp* infection, A,B: epithelial cell (×30,000, 17,000); C,D: parietal cell (×15,000, 30,000); E: chief cell (×17,000). F–H: PQD without *Hp* infection. F: epithelial cell (×15,000); G: parietal cell (×20,000); H: chief cell (×12,000)

of parietal cells aggregated in a chaos arrange (Figure 3F). The endoplasmic reticulum extended and aggregated, many edema mitochondria were presented and pyknosis nuclear membrane were deep staining in a lobular shape (Figures 3G and H).

For DHPW specimen without *Hp* infection, the microvilli of epithelial cell were relatively intact, and large number of mucus granules distributed (Figure 3I). However, the mitochondria of parietal cell increased, crowded and were edema in some of them, but distributed in a regular way, secretory tubule extended mildly, and pyknosis nuclear membrane with deep dying can be seen (Figure 3J). The rough endoplasmic reticulum extended obviously, and nucleus lobular was presented (Figure 3K).

### Subcellular Structure of PQD With or Without *Hp* Infection

For those PQD type specimen with *Hp* infection, sparse microvilli of epithelial cell and decreased mucus granules were shown, and *Hp* colonized among the microvilli (Figures 4A–E). The secretory tubule of parietal cell extended obviously even arrived at the membrane, with obvious fractured crests in the decreased mitochondria, and some of them were in vacuole shape. The rough endoplasmic reticulum was crowded and extended obviously in a pool-like way, with obvious pyknosis nuclear membrane.

For those PQD specimen without *Hp* infection, the microvilli of epithelial cell were relatively intact, but decreased in mucus granules. The amount of mitochondria decreased, and with a hoof-like shape, fractured crests with myeloid body presented in some of them. The rough endoplasmic reticulum of parietal cell extended in a pool-like way companied with a vacuole shape, aggregation with a sawtooth shape in the margin of nucleus membrane (Figures 4F–H).

## DISCUSSION

Our research showed that *Hp* colonization and mucus granules aggregation were the most obvious features in DHPW patients. The different characteristic of DHSD and DHSH syndromes showed mucus granules increased or destruction in the microvilli. Sparse microvilli and decreased mucus granules were

the characteristic of PQD patients with *Hp* infection. We speculate that a large number of mucus granules were induced to product by *Hp* attraction when destruction of the microvilli occurred, the aggregation of mucus granules was not only functioned as a swash for *Hp*, but also alleviated the damage of epithelial by regulating the $H^+$ flowback. In other words, the transformation of amount of mucus granules was the pathological results induced by *Hp* infection, and also the reflection of auto-protection of the organism, which shows the ability of organism to protect from evil-qi (*Hp*) in different syndromes, as was shown in our previous research.[7] DHPW with the characteristic of vigorous evil-qi is the most fierce stage for the struggling of healthy and evil-qi.

The mitochondria and endoplasmic reticulum are both the research highlights on the conjugation of structure and function, and the information transformation and function coordination impact the whole life of the cells.[8] Some researchers suggested that Pi was associated with mitochondria due to the similarity on the energy metabolism and macro-physical function of Pi in the charge of transportation and transformation.[9] The results in our research revealed, compared with the subjects without *Hp* infection, the mitochondria of the gastric mucosa in DHPW type patients with *Hp* infection were edema obviously, in irregular arrange, and with rough endoplasmic reticulum aggregation. For PQD patients with *Hp* infection, the mitochondria decreased, with fractured crests, vacuole accompany with myeloid body, rough endoplasmic reticulum extension in a pool-like way. Additionally, the pyknosis of nucleus membrane is the characteristics of healthy-qi deficiency and evil-qi lingering. Thus, we speculate that all the transformation of substructure is the microscopic reflection of the DHPW and PQD of HPCG.

In our experiment, specimen with positive results tested with RUT were screened with curved and spiral *Hp* dwelled in the surface of the epithelial cell and the deep of mucus layer under electron microscope, meanwhile, negative specimen were not observed, suggesting that the specificity of RUT and supermicro pathological morphology tested for *Hp* were identical. We speculate that it may be the result of our excluded criteria that patients who took anti-acid or antibiotics in the recent month were excluded, furthermore, all the specimens were biopsied in the antrum to avoid the divergence caused by the different location. However, based on a few samples, these results were limited.

## Conflict of Interest

The authors declare no conflict of interests.

## Author Contributions

Hu L and Lao SX designed the experiment. Li HY performed the experiments. Chen WQ translated the manuscript into English. Luo Q collected the specimens. All the authors had revised the manuscript and approved the final version.

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