UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
PhD STUDIES

PhD THESIS

IMPLICATIONS OF HELICOBACTER PYLORI BACTERIA IN GASTRO-DUODENAL PATHOLOGY. CLINICAL AND HISTOLOGICAL STUDY

ABSTRACT

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INTRODUCTION

Helicobacter pylori (HP) is the most important pathogenic agent, responsible for most gastric diseases. Discovered about 35 years ago (1983) by Marshall and Warren, this bacteria has become the most studied one, since multiple studies showed that the H pylori infection is a precursory condition for gastric and duodenal ulcers (Rozen P, 2004, Zhang C, Yamada N, Wu YL, et al, 2005; Kao CY, Sheu BS, Wu JJ, 2016). In 1994, H. pylori was recognized as being a well-defined carcinogenic by the International Agency for Cancer Research. H. pylori is responsible for 5.5% of gastric cancer cases worldwidely (Wroblewski LE, Peek RM, Wilson KT, 2010, Al Sayed A, Anand PS, Kamath KP et al, 2014).

The global prevalence of Helicobacter pylori infection is closely related to the socioeconomic status. Clinical and statistical data showed that HP infected almost 50% of the world population (Malnick SD, Melzer E,et al, 2014). The prevalence of H. pylori infection is variable in different countries; for example, a high prevalence is observed in the Latin America countries (75-83%), in contrast to a low prevalence in Japan (39.6%) and the USA (17.1%) (Calvet X, Ramírez Lázaro MJ et al, 2013). The prevalence among middle aged adults is over 80% in many developing countries, in comparison to 20-50% in developed countries.

The infection is acquired by oral ingestion of the bacteria and is transmitted intra familialy, mainly during first childhood (Ernst PB, Gold BD, 2000). It seems that, in industrialized countries, the direct transmission in humans through saliva, faeces, is dominant (Everhart JE , 2000; Uemura N, Okamoto S, et al, 2001; García A, Salas-Jara MJ, et al, 2014).

HP determines a permanent inflammation of the gastric mucosa in the infected persons (Malnick SD, Melzer E, Attali M et al, 2014). Still, the great majority of the infected population will not develop symptoms related to H. Pylori infection. This inflammatory response initially consisted in recruting neutophils, followed by T and B lymphocytes, plasmocytes and macrophages, with the alteration of the epithelial stratum.

The clinical progress of Helicobacter pylori infection is quite variable and it is influenced both by microbial factors and by host factors, as well. Patients with a prominent antral gastritis, the most common gastritis with Helicobacter pylori, are predisposed to duodenal ulcers, while the patients with gastritis of the gastric body

and multifocal atrophy are more prone to gastric ulcers, gastric atrophy, intestinal metaplasia and ultimately gastric carcinoma.

The H. pylori infection was also connected to Iron deficit anemia, B12 vitamin deficit and immune thrombocytopenic purpura (Kaptan K, Beyan C, Ural AU et al, 2000; DuBois S, Kearney DJ, 2005; Papagiannakis P, Michalopoulos C et al, 2013).

Chpater I. Histophysiology of the stomach

The stomach, the most dilated part of the digestive tract, is situated in the abdominal cavity, immediately under the diaphragm, in the upper abdominal part, projecting itself in the epigastrum and left hypochondrium. The area where the stomach is situated is called the gastric lodge and occupies the largest part of the left subphrenic region. It communicates with the other organs of the digestive tract, being interposed between the esophagus and small intestine, the esophagus separating limit being the cardia orifice, and the intestine separating limit being the pyloric orifice. In normostenic individuals, in orthostatism, the empty stomach has the form of letter „J", measuring approx. 18 cm long and 7 cm wide. When the stomach is full, its length may increase up to 25 cm, and its width up to 12 cm. The maximum capacity of the stomach in normal weight individuals is evaluated between 1 and 1.5 liters. The gastric wall is made of four concentric, superposed tunicae, namely: the mucous tunica, the submucous tunica, the muscular tunica and the serous tunica.

Gastric mucosa, the most important tunica of the stomach, lines the gastric wall. It continues the esophagus mucosa down into the duodenal mucosa. In the cardia of the esophagus Malpighian epithelium without keratinization, it is suddenly substituted by the gastric epithelium, which is a simple cylindric epithelium. The other structural elements of the asophagus wall continue in the stomach with similar structures, except for the adventicia, which is replaced by the peritoneal serosa. The gastric epithelium is a simple, monomorphus cylindric epithelium. It covers the entire surface of the gastric mucosa, including the gastric crypts. The gastric mucosa chorion is a lax conjunctive tissue, rich in blood vessels and numerous cells belonging to the immune system (lymphocytes, rare macrophages and mastocytes).

The submucosa is consituted from a lax conjunctive tissue, rich in conjunctive cells (lymphocytes, plasmocytes, mastocytes, polymorphonuclear eosinophils), fundamental substance, collagen and elastic fibers, blood and lymphatic vessels.

Muscles are formed of smooth muscular fibers on three layers: an external, longitudinal layer that continues the external layer of the esophagus muscles; a middle, circular, quite developed layer in the pyloric region, where it also forms the pyloric sphincter; a more developed internal inclined layer in the vertical part of the stomach. Among the fascicles of muscular fibers there is found little conjunctive tissue, blood vessels and the Auerbach nervous plexus.

The peritoneal serosa covers the stomach and is involved in its fixation to the organs in the abdominal cavity (liver, colon, diaphragm).

The stomach plays more parts in the process of food digestion: a mechanical one, a secretory one and an absorbtion part. The gastric juice, the exocrine secretion of the gastric mucosa, contains water, mucus, hydrochloric acid, pepsinogen, labferment, Castle's intrinsic factor (a glycoprotein secreted by the parietal cells, indispensable to vitamin 12 absorbtion), etc. The endocrine secretion is represented by serotonin and gastrin.

The gastric hormones regulate various important physiological functions of the stomach, including secretion and motility. The abnormal production of some of these hormones is associated to the development of various gastric diseases. However, various hormones (gastrin, somatostatin and ghrelin) and regulatory peptides are produced by the stomach cells.

One of the most important hormones secreted by the stomach is **ghrelin**. Ghrelin is a peptide hormone especially produced by the P/D1 cells located in the stomach when the stomach is empty and by the epsilon cells of the pancreas, stimulating apetite. It is very interesting that ghrelin acts on the same receptors of the hypothalamic neurons on which leptin acts, as well, known as an anorexigenic hormone. The stomach also produces low quantities of **leptin** anorexigenic hormone, also the main source of leptin is the adipose tissue.

Another main hormone produced by the stomach is **gastrin**, which continues to be investigated and involved by gastroenterologists in order to associate it to various gastrointestinal diseases.

CHAPTER II. Helicobacter pylori – microbiological aspects

Helicobacter pylori is the main bacterial carcinogen officially acknowledged. *H. pylori* infection represents a key factor in the etiology of various gastrointestinal diseases, varying from chronic active gastritis, with no clinical symptoms, to

gastroduodenal ulcer, gastric adenocarcinoma and lymphoma of the lymphoid tissue associated to mucosa.

The members of *Helicobacter* class are microaerophilic organisms and, in many cases, they are catalase and oxidase positive, lots of them also being urease positive. Gastric *Helicobacter* species adapted to the unhospitable environment found in the gastric mucosa, at present being considered that the stomach of all mammals may be colonized by members of the *Helicobacter* class. All the known species of *Helicobacter* gastric are urease-positive and have a high motility with the flagellae. Urease increases their short term survival in a strongly acid environment of the gastric lumenus, its motility making easy the raid movement towards the environment with a pH closer to neuter of the gastric mucosa.

H. pylori is a Gram-negative bacteria, measuring from 2 to 4 μm in length and from 0.5 to 1 μm in width. Although it has a usual convolutional form, the bacteria may also appear as a bar, while the coccoid forms appear after a prolonged in vitro growth or as a result of an antibiotic treatment. The bacteria has from 2 up to 6 unipolar non-capsulated flagellae, of an approx. 3 μm length, which give it motility and allow a rapid blood flow in viscous liquids, such as the mucous layer that lines the gastric epithelium.

H.pylori is a particular microorganism that requires complex growth environments. Lots of times, these environments are supplied with blood or serum. In culture environments, *H. pylori* forms small, translucid colonies (1-mm).

H. pylori is a microaerophilic bacteria that does not tolerate high oxygen concentrations, but it requires at least 2% O_2.

The general composition of the cellular covering of *H. pylori* is similar to that of other Gram-negative bacterias. It consists of an internal membrane (cytoplasmic), periplasm with peptidoglycans and an external membrane. The external membrane is formed of membranary phospholipids and lipopolysaccharides. The external membrane of *H. pylori* contains cholesterol glucosides, quite rarely found in bacteria. In the *H. pylori* genome there are codified a large number of external membrane proteins.

The dimension of the two genomes of *H. pylori* discovered is about 1.7 Mbp, with a content of C+G from 35 to 40%. The genome includes 1491 and 1587 genes, respectively. Lots of roots wear one or more cryptic plasmides, which do not seem to bear resistance genes to antibiotics or virulence genes.

The rapid responses to the stressing changes of environment conditions are often mediated through transcription changes of the gene sets that codify some factors involved in the relations with these stress factors. Such examples are the expression of defence genes against oxidative stress, as a response to the oxidative stress. In many bacteria, these systems that respond to stress are often codified on genes organized in an operon, and the transcription is regulated by one or two regulatory proteins.

CHAPTER III. Clinical aspects of conditions associated with *H. pylori*

Acute and chronic gastritis. H. pylori colonization almost always leads to infiltration of neutorphils and mononuclear cells of the gastric mucosa, both antrally and at H. pylori body level. It especially colonizes the gastric antrum, where there are present only few parietal cells that secret hydrochloric acid. The subjects in whom the acid secretion is affected by any mechanism, have a more balanced bacterial distribution between antrum and the body.

Peptic ulcer. Gastric or duodenal ulcers (called peptic ulcer) are defined as defects of the mucosa, with a diameter of at least 0.5 cm that go inside the mucosa muscles. Both gastric and duodenal ulcers are strongly related to H. pylori. In the initial reports all over the world, in the first decade after discovering H. pylori, approximately 95% of duodenal ulcers and 85% of gastric ones occurred in the presence of H. Pylori infection. More studies estimate that the risk for ulcerous disease in subjects with positive H. Pylori is from 3 up to 10 times higher than in the subjects with negative H. pylori. Developing ulcer in the presence of H. pylori is influenced by a variety of factors related to the host and the bacteria. The ulcer occurs, most of the time, in areas where the mucosa inflammation is the most severe.

Non-ulcerous dyspepsia is defined as the presence of symtpoms of upper gastrointestinal discomfort, with no structural changes, identified during diagnosis, particularly during upper gastrointestinal endoscopy. The dyspepsia symptoms may have a gastro-esophageal reflux characteristic, with epigastric burns and regurgitations; there may appear as symptoms of motility alteration, with early satiety and nausea; other symptoms may be similar to ulcer (pain and vomiting). 30% up to 60% of the patients with functional dyspepsia present a H. Pylori infection.

Atrophic gastritis, intestinal metaplasia and gastric cancer. Chronic inflammation induced by *H. pylori* may lead to the loss of normal gastric mucosa

architecture, with destroying gastric glands and replacing the intestinal epithelium by fibrosis. This process of gastric atrophy and intestinal metaplasia takes place in approx. half of the patients colonized with H. pylori, mainly in the patients with a more severe inflammation. Based on some multicentered studies, there was estimated that colonizing H. pylori increases about 10 times the risk for gastric cancer.

CHAPTER IV. Importance of research. Proposed objectives

For more than one century, gastric pathology was explained by the disbalance between the aggressive and protecting factors of the gastric mucosa. After the ’80s, when two Australian doctors, Marshall and Warren discovered the Helicobacter pylori bacteria and made connections between the infection Helicobacter pylori (H pylori) and gastric pathology, the physiopathological mechanisms of the main gastric conditions suffered significant changes. From that moment on, most evidence supported the idea that H pylori infection is an early condition for gastrities, gastric and duodenal ulcers, and even gastric cancer. The evaluation of H. pylori infection performed in various academic clinics, research centers, hospitals, gastroenterology consultories were a continuous concern, taking into consideration the high number of patients with gastroduodenal symptoms and the high number of patients infected with H. pylori. Our research is part of the the present research on the gastroduodenal pathology.

Proposed objectives:

- evaluation by retrospective clinical and statistical studies of the gastroduodenal pathology and H.pylori infection;
- evaluation of various risk factors regarding the gastroduodenal pathology;
- evaluation of gastric histopathological changes in the patients with positive H.pylory.

CHAPTER V. Clinical and statistical study of the gastroduodenal pathology within the Emergency Hospital of Tg. Jiu.

The study included a group of 5793 patients admitted to the departments of Internal Medicine and Surgery within the Emergency Hospital of Tg. Jiu between January 1st 2008 – December 31st 2012. The clinical and statistical analysis of the studied group comprised: sex and age of patients, social environment (urban, rural), gastric lesions, presence of tumoral lesions as well as the histopathological type of

the lesion. Collection of clinical and paraclinical data was performed from the observation sheets, the Hipocrate informatic system, surgical protocols, laboratory tests sheets and pathological anatomy records. For highlighting some important clinical aspects, these data were processed in Excel and transposed in charts for performing more suggestive images of this lesion.

In our study, the gastroduodenal pathological evaluation showed that this is relatively easy distributed by sex and social environment. Still, males were more affected than females, and the gastric pathology was more dominant in the rural area than in the urban one.

Gastric ulcer represented a total of 458 patients admitted during the 5 ani years of study. In this condition, there was observed a significant reduction of the cases during the studied period. If in 2008 there were hospitalized 117 cases, in 2012 there were only 51 cases.

The number of patients with duodenal ulcer was about 3 times higher than that of gastric ulcer, being recorded a number of 1360 patients. In this pathology, too, there was recorded a descending trend, thus, in 2008 there were hospitalized 457 patients and in 2012 only 151.

Regarding gastrites, in the 5 years of study, their number was of 2566 cases. Year distribution varied from 340 to 721 cases.

Gastric tumors, mostly adenocarcinomas, totalized a number of 750 cases. Their tendency varied quite a little from one year to another, its values being included between 134-166 cases every year.

CHAPTER VI. Study of H. pylori infection in the gastroduodenal pathology

The study included a group of 1525 patients that presented to Renasterea Medical Healthcenter of Craiova between 2010-2014 for various complaints of the upper digestive tract. In this medical healthcenter, the patients underwent a clinical and endoscopical examination. The determination of H. pylori infection was performed directly through the urease test and indirectly by testing the anti-helicobacter pylori antibodies from the blood serum.

Of the total of 1525 patients, 1089 (71.41%) came from the urban area, and 436 (28.59%) came from the rural area. The presence of a higher number from the urban area may be due to a more easy access to medical services, social, financial

and professional status of the patients, as well as to a higher medical education in comparison to those from the rural area.

Of the studied group, 971 patients (63.67%) were infected with H. pylori, and the rest of 554 (36.33%) were not infected. Our data confirm the fact that the persons infected with H. pylori in Romania exceed 60%.

By evaluating the association of H. pylori infection with the esogastric infection, there was observed that the difference between the patients from the rural area and the ones from the urban area was not a significant one. Thus, in the urban area, there was found H. pilori+ a percentage of 62.63% of the patients, while in the rural area there were 63.65% of H. pylory+. This unsignificant difference showed that, overall, the H. pylori infection is spread in the same prorportion both in the rural and urban areas.

The age of persons with esogastric pathology varied from 16 to 87 years old. The most affected by this pathology were the ones in the age group 50-59 years old. The H. pylori infection in the studied group had approx. the same incidence in the age groups 30-39 years old, 40-49 years old and 50-59 years old, respectively. This aspect shows a relatively strong infection with H. pylori unde the age of 30 years old.

CHAPTER VII. Histopathological aspects of gastric lesions in the H.pylori+ patients

The histopathological study was performed on a number of 79 biopsy gastric pieces, endoscopically harvested, and on 43 stomach fragments harvested from some patients infected with H. pylori, who required a surgery, either for gastroduodenal ulcer or for gastric tumors. The biological material was fixed in 10% formalin and processed by the classical histopathological technique of paraffin inclusion. The classical histopathological stainings allowed us to highlight the presence of important lesions in the gastric wall, starting from chronic gastrites with incomplete or complete intestinal metaplasia, atrophic gastritis, hemorrhagic gastritis, chronic gastric ulcer and even gastric malignant lesions (poorly, moderately and well differentiated adeocarcinomas, muciparous carcinomas, gastric lymphomas).

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