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MODERN METHODS OF DIAGNOSIS AND TREATMENT OF HELICOBACTER PYLORI

In this review the modern data about the history of discovery, biology, virulence factors, infection, pathogenesis and modern methods of diagnostics of gram-negative, mobile bacteria Helicobacter pylori (H. pylori), being a major cause of chronic gastritis and peptic ulcer disease are having been investigated. H. pylori inhabits the surface epithelial cells of the gastric mucosa of the stomach and part of under-pyloric. H. pylori is a bacterium with numerous cilia located at both poles. It has virulence factors allowed breaking the body's natural defense. H. pylori infection spreads by the faecal-oral route. H. pylori infection plays an important role in the pathogenesis of gastric and duodenal ulcers, gastric cancer or gastric lymphoma type mucosa associated lymphatic tissue (MALT). The diagnosis of H. pylori may be invasive included an urease test and bacterial cultures, and non-invasive – a serological test, breathing tests, testing of stool antigen Hp.

Key words: H. pylori, virulence factors, adaptive characteristics, pathogenesis, methods of diagnosis, medicines dosage.

Introduction

Helicobacter pylori (H. pylori) is a gram-negative bacterium, known to sticks. It inhabits the surface epithelial cells of the gastric mucosa and in the under-pyloric part of the stomach. According to the World Health Organization (WHO) in developing countries, 70% of human population is a carrier of *H. pylori* without any symptoms. Infection occurs most often between 40–50 years of age. A carrier is not so common in the developed countries (according to WHO, 30% of the population) [1]. Infection with this bacterium concerns in Poland is 84% of adults and 32% of children and adolescents up to 18 years of age [27]. The fact that there are so many carriers of *H. pylori* without any disease symptoms is allowing a microbiologist to suggest that we should consider this bacterium as a representative of the physiological biota of the stomach [2]. The bacteria *H. pylori* were discovered in 1875 by German scientists, but they failed to breed them artificially in the laboratory and quickly forgot about them [3]. Once again, regardless of the German research scientists, these bacteria

were discovered in 1899 by Walery Jaworski from the Jagiellonian University. He observed the characteristic spirals of bacteria and named them *Vibrio rugula*. At first he suggested that they can cause stomach disorders. His observations were published in the "Handbook of diseases of the stomach", but they went unnoticed. The organism was grown in 1982, then also they noticed the high degree of morphological similarity to the family *Compylobacter*. Initially, in 1984 it was named the bacterium *Compylobacter pyloridis* that in 1987, changed it into *Compylobacter pylori* [4]. After researching the genome in 1989, the strains were eventually qualified as *Helicobacter*. The discovery is attributed to two Australian pathologists at the University of Perth, Barry Marshall and Robin Warren, who with this discovery were awarded the Nobel Prize in 2005 in the medical field [5].

Morphology and physiology of *Helicobacter pylori*

H. pylori is a mobile, spiral microaerophilic bacterium, forming a tight usually three rolls [6]. Morphologically similar to *Campylobacter*, although they are generally larger. The length of the bacteria is in the range of 3–6 microns and a width of 0.6 microns. The microorganism does not produce spores. It is characterized by the presence of numerous cilia on both ends [8].

The bacterium exists under small amount of oxygen on the surface of the epithelial cell lining of the stomach and duodenum, less the membrane lining of the esophagus. It has high motility in the mucus covering the mucous membranes. Mobility is the result of the aforementioned, a number of cilia, usually six per each pole. H. pylori produces hydrogenase – the enzyme, which enables to extract energy from the oxidation of hydrogen molecules (H^+) produced by other intestinal bacteria. It can also move from the spiral form to cocci, which probably facilitates its survival and proliferation [8]. *H. pylori* is sensitive to external conditions, however, it can survive in the acidic environment (pH of the stomach 2–4). It is characterized by five major types of outer membrane proteins: adhesins porins, iron transporters, proteins linked to the cilia and protein of unknown function. Like other Gram-negative bacteria, the outer membrane of *H. pylori* consists phospholipids and lipopolysaccharide. The outer membrane has also glucosides cholesterol, which exists in a small number of other bacteria. Biochemical properties can be used to differentiate bacteria in the genus [9].

Virulence factors of *Helicobacter pylori*

Virulence factor defines the characteristics of the bacteria that allow it to overcome the natural defenses of the body. *H. pylori* lives in unfavorable environment, which is the human stomach. The stomach content has a very low pH 2–4, which for the majority of bacteria is fatal. The characteristics for survival of *H. pylori* in such a very difficult environment are: (1) urease – production of large quantities of this enzyme, allows the *H. pylori* decomposition of urea to ammonia and carbon dioxide. The final product, which is ammonia, neutralizes the acidic gastric juice and increases the pH in the immediate vicinity of the bacteria. (2). Cilia – cilia pole arrangement allows movement of bacteria and penetrates the mucous layer of the

stomach. The mucous layer protects the epithelial cells lining the stomach by the action of hydrochloric acid bacteria thus uses the host defense system. Cilia are composed of two flagella: FlaA and FlaB. (3). The pump is pumped H^+ ions from the cells. Locking these pumps by some drugs raises the pH in the stomach. (4) System antioxidant – neutralizes free radicals generated by the immune system cells – neutrophils. It consists of: catalase, superoxide dismutase, protein MdaB and Napa and an efficient system of repairing DNA. (5) Adhesins are responsible for the adhesion of bacteria to the gastric epithelium. (6) Cytotoxins – vacA genes encoded in (50% of strains) and cagA (70% strain). Vacuole toxin (vacA) causes epithelial cells to the fusion of the endosomes with lysosomes and promotes the formation of large vacuoles. Facilitates the free flow of urea to the stomach. The toxin disrupts the cytoskeleton of epithelial cells and enhances the adhesion of bacteria to damaged epithelium [1, 10–12].

Infection and pathogenesis

The infection spreads by the faecal-oral route and is associated with poor hygiene and low standard of living. Numerous virulence factors allow *H. pylori* colonization of epithelial cells and cause the pathological changes [6]. *Helicobacter pylori* infection plays an important role in the pathogenesis of gastric and duodenal ulcers, gastric cancer and gastric lymphoma type mucosa associated with lymphatic tissue (MALT). Defensive reactions of the body caused by infection are not able to eliminate bacteria. This bacterium is causing 80–90% of cases of gastritis [6]. Epithelial damage is a result of substance affected produced by bacteria, such as amonia, protease, cytotoxin A, and some of phospholipase are making epithelia damage [13]. Unfortunately, in most cases there is a chronic infection phase, which is the most common cause of gastritis. In 80% of cases there are no obvious symptoms of the disease. The level of gastrin and acid secretion are correct. 15% indicates the production of large amounts of hydrochloric acid led to inflammatory changes within the under-pylorus of the stomach. This in turn causes the secretion of gastrin enhanced by increased secretion of hydrochloric acid, and thus the possibility of ulcers of the stomach and duodenum. In the remaining 5% of the cases the infection causes changes in the gastric mucosa within the body and fundus. Chronic gastritis associated with *H. pylori* infection is causing atrophy of the mucous membrane and the occurrence of outbreaks of intestinal metaplasia [17]. Most people suffering from peptic ulcer disease (80-90%) have an infection with *H. pylori*. Underlying ulceration it may have several mechanisms, depending on *H. pylori* infection: increased production of hydrochloric acid, the formation of foci of gastric metaplasia, the development of local inflammatory reaction mucosal defense whether the reduction process of mucosa [13]. Gastric cancer is characterized by two types: the intestinal and diffuse [14]. *H. pylori* is known as a cause of gene mutations leads to loss of the primary function of gastric epithelial cells and their uncontrolled growth and unlimited division. Well documented is the effect of chronic *H. pylori* infection on the development of intestinal form of cancer, without affecting the other morphological type of cancer [21]. Prolonged stimulation of the immune system in *H. pylori* infection results in a risk of lymphoma of gastric

mucosa. The bacterium has an effect on the development of 90% stomach MALT lymphoma. The risk of the occurrence of this condition is higher when the bacteria present in the genome gene VacA [15] Infection with *H. pylori* is not without effect on the absorption of medicines. It was found necessary to administer higher doses of thyroxine in the patients with hypothyroidism, in which there is concurrent infection with *H. pylori* [25]. *H. pylori* also affects the immune system response in vitro by activating B cells, followed by the production of antibodies, T cell activation, activation of macrophages, neutrophils and their chemotactic activity [26].

Diagnosis of *H. pylori* infection

A prerequisite for the initiation of treatment is to identify infection. Diagnostics, depending on the performance of endoscopy, can be invasive or non-invasive [16]. The decrease in the accuracy of the test may emerge the use of medicines, that is why it is necessary to discount for 7–14 days before the planned screening of *H. pylori*. Medicines that can affect the accuracy of the study are: medicines with proton pump inhibitors, the drugs from the group of H^+-blockers and antibiotics. The kind of method of diagnostic *H. pylori* is this that requires endoscopy with biopsy. The downloaded material (approximately 5 mm) may be tested for the production of urease, histological evaluation or assumption of culture [16]. Cuttings are taken from the pylorus, less of the cardiac and fundus (the material is taken when there was a shift towards the top of the stomach colony, which is a consequence of taking proton pump inhibitors). The rapid urease test involves placing a previously downloaded a piece of the lining of the stomach to the paper soaked in a solution of urea. If the material is *H. pylori* bacteria it reaches the decomposition of urea to ammonia, thereby raising the pH and the change in color paper. The test has a high specificity (> 90%) and sensitivity (> 90%). Reading is possible after 15 minutes [17]. The investigation has two main advantages. Firstly, you conduct research on antimicrobial sensitivity. Secondly, the material can be characterized in detail. The sensitivity of laboratory cultures of *H. pylori* is 95%. In addition, breeding *H. pylori* requires special conditions. It requires micro-aerobic environment, high humidity, and the incubations are 35–37 °C for 7–10 days. Positive cultures were detected after 3–5 days of incubation. Such method of diagnosing the organism is associated with high costs [18]. Histological evaluation involves staining of *H. pylori* with hematoxylin and eosin (H & E). The material for dyeing is taken from the stretch of the gastric mucosa. H & E staining, however, may be unreliable, as in the formulation have different bacteria, as this dye is not specific for *H. pylori*. A better way is to identify the staining of *H. pylori* by Warthin-Starry staining with Giemsa and modified. Histological identification of bacteria with characteristic morphology *H. pylori*, is partly based on the observation. The factors that affect the ability to correctly identifying are: the density of colonies of bacteria, types of dyeing and experienced analyst. Sensitive staining technique is the connection of methods of H & E staining, silver Steiner and blue – a method of Genta. Staining is evident bacteria *H. pylori*, and also enables the assessment of the histology of the stomach,

which eliminates the need for different coloring. This procedure may, however, be difficult for the technical execution [18]. Non-invasive diagnosis of *H. pylori* include: urea breath test consisting of administration to the patient to drink a solution of urea labeled carbon ^{13}C or ^{14}C. In the event of possible *H. pylori* infection there is a distribution of the labeled urea by urease, and bacteria formed isotopically labeled carbon dioxide eliminated from the body of the exhaled air. With sensitivity and specificity of this method there are estimated at 95% accurate, this method compares favorably to other similar diagnostic procedures to detect *H. pylori*. Before starting the test, carefully brush your teeth, tongue and throat surface, because the normal flora that lives there can produce urease and generate false positive. Due to the costs and technical difficulties urea breath test is not widely used [19]. Urinary urea breath test involves administering to drink the solution of urea labeled with an isotope of nitrogen. When the existence of *H. pylori* infection in the stomach urea decomposes to carbon dioxide, ammonia is labeled. Ammonia enters the circulation and is rapidly excreted by the kidneys. If the test is positive, this means that present in the urine will be labeled nitrogen. The result is obtained in 12–15 hours [20]. Reliability of both urease tests is similar. There is also another method of labeling atoms in urea. Part of the stripe-labeled carbon dioxide is passed into the bloodstream, and the labeled carbon is determined by a blood test, but not exhaled air [19]. There are two methods for labeling carbon (^{14}C or ^{13}C), with which the method of ^{13}C-labeling. While the first method is cheaper and does not require specialized analyzer, but may be more dangerous for the patient because of the biological half-life in living organisms carbon is short, so there is a potential risk of irradiation of the body [19]. The PCR involves the proliferation assay for bacteria-specific DNA fragment encoding the toxin – vacA and cagA. Typically, the test sample is feces. The sensitivity of the test for the presence of DNA in the material-specific H. pylori is judged to 50–60%. The genome of the bacteria is also present in the saliva or the sensitivity of the assay is very low (about 25%). The advantages of PCR tests have got very high specificity [21]. Another method for diagnosing *H. pylori* infection is ELISA. The principle of the test consists in the fact that the antibody bound to a specific enzyme can specifically recognize the protein (contained in the membrane of *H. pylori*), which has been previously immobilized. After administration of the antibody immune complex is formed, which resulted in antibody immobilization. After addition of substrate the enzymatic reaction takes place and new product is formed. Presence of protein in tested material is resulted in formation of colorful product from colorless subtrate [24] Another research method is to study the presence of *H. pylori* antigens in stool (HpSA-pylori Stool Antigen). This assay is non-invasive, sensitivity and specificity are similar to the test tract (approximately 90%). It is particularly useful in the diagnosis of *H. pylori* infection in children. Nowadays it is not available in Poland [27]. The best method to be used to diagnose *H. pylori* infection is the above-described non-invasive breath test that is fast, easy and has a high sensitivity and specificity (> 90%). Top-breath test to confirm the result of serological tests to assess the levels of IgM, IgG and IgA in the serum of patients.

Treatment of *H. pylori*

The target of treatment is to completely remove bacteria embedded in the gastric mucosa known as eradication. The vast majority of people with asymptomatic *H. pylori* infection does not apply it routinely. In case of ulcers caused by *H. pylori* the infection early eradication and prevention of relapse in fact leads to permanent cure the patient [23]. Since the use of antibiotics against *H. pylori*, it was observed that monotherapy – one antibiotic using – does not provide a complete therapeutic success, despite the sensitivity of *H. pylori* in vitro to a variety of antibiotics. The consensus achieved in Maastricht in 1997 was recognized as an effective therapy, combinations of three medicines for seven days: proton pump inhibitor (PPI), metronidazole or tinidazole and amoxicillin. The second consensus – Canadian in 1999 introduced additional clarithromycin, an antibiotic was used interchangeably alongside metronidazole and amoxicillin [23]. According to that, it should be used: the first-line medicines, so-called triple therapy: bismuth salts or proton pump inhibitor and clarithromycin and metronidazole or amoxicillin, second-line medicines, so-called quadruple therapy, include salts of bismuth, a proton pump inhibitor, metronidazole, tetracycline. Triple therapy is effective in 80–90% of cases, unless it is carried out among the strains with high sensitivity to antibiotics. The most common used in treatment are the aforementioned antibiotics and chemotherapeutics, and fluoroquinolones, as well as proton pump inhibitors: omeprazole, pantoprazole, lansoprazole, rabeprazole, esomeprazole [23]. The problem of treatment *H. pylori* infection is an infection, as well as the increasing resistance of bacteria to standard medicines. The strains in Poland are the most resistant to metronidazole and clarithromycin. The resistance to antibiotics is common and often is caused by chronic using of medicines. This resistance is common in the developed countries, where resistance to clarithromycin is in the US 10–12.5%, in Canada below 4%, and in Europe, depending on the region – 4.2% northern, eastern, 9.3%, and in the south up to 18%. It is assumed that regardless of the type of used antibiotics the treatment should last for 1–2 weeks. The chances of successful eradication decline, after two attempts unsuccessful clarithromycin treatment, up to 60%. This demonstrates the presence of medicines-resistant strains of *H. pylori* to antibiotics and chemotherapeutic agents, considering the introduction of standard quadruple therapy or sequential therapy due to the increasing resistance of *H. pylori*. Sequential therapy involves the use of proton pump inhibitor and amoxicillin of 1.0 mg for 5 days and for the next 5 days proton pump inhibitor, clarithromycin and tinidazole in doses of 2 x 500 mg [22]. It should be marked that in addition to a high rate of resistance of *H. pylori* to the medicines, adversely affect the performance of antibiotics has also the low pH of the stomach, which inactivates the antibiotics, and thus complicates treatment [22].

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СУЧАСНІ МЕТОДИ ДІАГНОСТИКИ І ТЕРАПІЇ *HELICOBACTER PYLORI*

Реферат

В огляді представлені сучасні дані про історію відкриття, біологію, чинники патогенності, інфекційний процес та сучасні методи діагностики грамнегативних рухливих бактерій Helicobacter pylori – одного з основних збудників хронічного гастриту та виразкової хвороби. Helicobacter pylori заселяє поверхню епітеліальних клітин слизової оболонки шлунка та частини пілоричного відділу. Інфекція, яку викликає Helicobacter pylyori, сприяє розвитку і визначає патогенез виразки шлунка або низькодиференційованої лімфоми шлунка (MALT-лімфоми). В діагностиці Helicobacter pylori використовують як інвазивні (уреазний тест і вилучення збудника), так і неінвазивні (серологічний та дихальні тести, визначення Нр-антигенів в калі) методи.

Ключові слова: Helicobacter pylori, чинники патогенності, адаптивні характеристики, патогенез, методи діагностики, дозування препаратів.

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СОВРЕМЕННЫЕ МЕТОДЫ ДИАГНОСТИКИ И ТЕРАПИИ *HELICOBACTER PYLORI*

Реферат

В данном обзоре представлены современные данные об истории открытия, биологии, факторах патогенности, инфекционном процессе и современных методах диагностики грамотрицательных подвижных бактерий – Helicobacter pylori – одного из основных возбудителей хронического гастрита и язвенной болезни. H. pylori заселяет поверхность эпителиальных клеток слизистой оболочки желудка и части привратника. Инфекция, вызванная H. pylori, способствует развитию и определяет патогенез язвы желудка и двенадцатиперстной кишки, рака желудка или низкодифференцированной лимфомы желудка (MALT-лимфомы). В диагностике H. pylori используют как инвазивные (уреазный тест и выделение возбудителя), так и неинвазивные (серологический и дыхательные тесты, определение Нр-антигена в кале) методы.

Ключевые слова: Helicobacter pylori, факторы патогенности, адаптивные характеристики, патогенез, методы диагностики, дозировка препаратов.

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