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Molecular Genetic Study of Helicobacter Pylori in Rheumatological Patients

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Annotation: A review of a molecular genetic study for the diagnosis of the genotypic features of Helicobacter pylori in the formation of NSAID gastropathy in rheumatological patients is presented, allowing the practitioner to interpret the results obtained and correct eradication therapy.

Keywords: Helicobacter pylori, diagnostics.

Ongoing research in developing invasive and non-invasive methods for diagnosing H. pylori infection will significantly improve the treatment of diseases associated with this microorganism. Although the H. pylori bacterium has been unanimously declared a human pathogen, only a small proportion of infected people develop an associated disease, which is mainly due to the presence of H. pylori virulence factors. Based on the research currently being conducted, individual identification of bacterial virulence factors is used to stratify the risk of developing a particular pathology, and the issue of risk assessment in combination with histopathological assessment of gastritis is discussed.

There are generally accepted diagnostic methods for detecting H. pylori infection and checking virulence factors. Nevertheless, although the gold standard methods are still mainly based on biopsy methods, the high prevalence of infection, especially in areas with low levels of medical care, suggests an urgent need to introduce non-invasive and, preferably, inexpensive diagnostic methods with high throughput. The choice of such a diagnostic method depends on the sensitivity, specificity, accessibility, complexity, cost, and speed of obtaining results [1]. Unfortunately, none of the methods currently used fully satisfy these criteria. Although biopsy-based methods have very high specificity, ponies have relatively low sensitivity, mainly due to factors that are difficult to control, such as the location of the biopsy sample. Even though all the rules for proper sampling are followed, other factors, such as the use of medications immediately before the study, may affect the growth or presence of detectable H. pylori.

The PCR-based diagnostic method has a slight advantage over other methods [2,3]. As a highly sensitive and specific method, PCR is applicable for detecting H. pylori infection and monitoring the effectiveness of treatment and therapy. The studies that were conducted have shown that with proper PCR design, one copy of genomic DNA is sufficient to obtain a positive result indicating the presence of H. pylori in the sample. Moreover, DNA samples isolated from various sources, such as gastric biopsy, oral cavity samples, and stool samples, can be analyzed by PCR. Accordingly, excluding possible chances of contamination, PCR can be used as the gold standard. The main disadvantage of PCR is the level of diagnostic clinics and the introduction of this method in regions with poor medical care.

Since other biopsy-based methods have several method-specific disadvantages, such as the need to be equipped with endoscopic equipment or additional systems for rapid urease testing, they are unsuitable as gold standards. Most efforts to implement an adequate gold standard have recently focused on non-invasive methods. In addition to PCR, serological methods for detecting specific H. pylori antigens in various samples have been significantly improved. Bioinformatic analysis helps in choosing the optimal antigen used in serological analyses. Thanks to the selection of a suitable antigen and the application of advanced nanotechnology techniques in the development of tests, new serological tests have recently achieved relatively ideal results [11]. Due to serological examination, antigens were identified in all strains of H. pylori, which have not only high immunogenicity but also cause specific

antibodies that disappear after a relatively short time. Improved and simplified methods for detecting specific antibodies, such as linear analysis and rapid tests, have significantly improved the use of serological tests to detect H. pylori. These approaches cover most of the criteria mentioned for H. pylori diagnostic tests. They are applicable not only to high-performance studies of large cohorts but also to medical personnel because they are very economical, easy to analyze, and understandable.

It should be noted that there is obvious evidence that not every strain of H. pylori is harmful and requires treatment [4, 5]. In addition, data on the positive aspects of H. pylori infection for the human host are accumulating [6]. Therefore, risk stratification based on epidemiological data is strongly recommended. Such assessments are currently possible only with the help of serological analyses, which may indicate infection with pathogenic strains of H. pylori.

To date, numerous studies have been conducted to study the effect of the molecular and genetic composition of H. pylori and its pathogenic factors on the clinical course of gastroduodenal diseases. Currently, much attention is being paid to the study of the genotype of H. pylori, which causes stomach cancer, gastritis, and gastroduodenal ulcers. Several researchers have shown a link between H. pylori genes and the development of gastric ulcer disease; such genes include CagA, vacAm1, vacAm2, vacAs1, vacAs1a, vacAs1b, vacAs1c, vacAs2, babA, iceA1, iceA2 and dupA [13]. The second most crucial factor of atherogenesis in Helicobacter pylori infection is the use of nonsteroidal anti-inflammatory drugs (NSAIDs). However, the effect of H. pylori pathogenic factors on erosion and NSAID-induced gastric and intestinal mucosa has not been sufficiently studied. The results of studies on the effects of H. pylori genes CagA, vacAm1, vacAm2, vacAs1, vacAs1a, vacAs1b, vacAs1c, vacAs2, babA, iceA1, iceA2, and dupA on the likelihood of NSAID-induced gastropathy in patients with rheumatoid arthritis (R.A.) have shown that the listed H. genes pylori can be considered as additional risk factors for NSAID gastropathy [15].

A number of other researchers came to similar conclusions. For example, genetic analysis of H. pylori in East Asia and South America has shown that the presence of the dupA gene creates a risk of duodenal ulcer and reduces the risk of atrophy of the gastric mucosa, intestinal metaplasia, and stomach cancer. In gastritis, the dupA gene was found in 21% of the examined patients and patients with duodenal ulcers - in 42% of cases [5]. In Brazil, the H. gene pylori was detected in 41.5% of dupA and kagA in 47.8% of patients with symptoms of dyspepsia; in some cases, a combination of dupA, CagA, and vacAs1/m1 genotype was observed, which, according to the authors, is associated with gastrointestinal disease [7].

Brazilian researchers, in turn, noted that during the study, the iceA2 and CagA H. pylori genes were often encountered in patients with peptic ulcer disease, and the presence of the babA2 H. pylori gene did not lead to gastroduodenal disease [8]. Y.H. Chang et al. It has been shown that the vakAm2 + H. pylori genotype is associated with developing gastroduodenal ulcers [14]. In another study in the Republic of Belarus, the detection rate of the H. pylori CagA gene in patients with gastric cancer was 68.2%, in patients with gastritis - 61.6%, peptic ulcer - 72.7%, duodenal ulcer - 87.5%, and in the control group - 55.6% [9].

The use of H. pylori and NSAIDs is an independent risk factor for erosive and ulcerative development of the mucous membrane of the gastroduodenal zone [10]. There are separate studies of the effect of the molecular and genetic composition of H. pylori on the development of gastroduodenal pathology with prolonged use of NSAIDs. Some studies have shown that CagA-positive strains of H. pylori double the frequency of bleeding when taking NSAIDs [5]. Other researchers confirmed their data, noting the presence of CagA and H. strains. pylori increases the frequency of gastrointestinal and duodenal bleeding in people taking low doses of aspirin [10]. Gastro- and duodenopathy associated with nonsteroidal anti-inflammatory drugs (NSAIDs) and H. pylori infection are currently considered two independent pathogenetic factors of gastrointestinal tract diseases capable of potentiating each other. That is why carrying out a timely diagnosis of H. pylori is essential for the correct choice of further treatment tactics.

The diagnosis of genetic risk factors for NSAID gastropathy in patients with rheumatoid arthritis infected with H. Pylori is carried out to determine the effect of CagA, vacAm1, vacAm2, vacAs1, vacAs1a, vacAs1b, vacAs1c, vacAs2, babA, iceA1, iceA2 and dupA genes of H. pylori on the possibility of gastropathy induced by prolonged use of NSAIDs (NSAID-gastropathy).

To identify the presence of genes, diagnosis is carried out in two stages: the first is isolation and identification, and the second stage is the preparation of genomic DNA and PCR analysis. At the first stage, it is necessary to obtain endoscopically three biopsies in the area of large curvature of the antrum of the stomach, two of which are used for histological examination and one for H. pylori cultivation. Gastric biopsy samples for cultivation are stored in a transport medium consisting of thioglycolate with 1.3 g/l agar, with 3% yeast extract, and are necessarily delivered to the laboratory on the same day.

Next, the second stage is carried out: the preparation of genomic DNA and polymerase chain reaction (PCR) analysis. DNA from each H. pylori isolate is extracted using a special kit. The genotypes of the s-region vacA (s1 or s2) and m-region (m1 or m2), the presence of the genes CagA, cagE, oipA, iceA, and babA2 are determined using specific primers. The CagA genotype, the East Asian type (type 1a) or the Western type (type 2a), is also determined by PCR, as described earlier [12].

The game gene (urea) is used as a control for detecting H. pylori DNA. All studied PCR mixtures are prepared in a volume of 25 ml containing 1 PCR buffer, 500 nM of each primer, 1.5 mM MgCl2, 200 mM of each dNTP, 1.5U Taq DNA polymerase, and 300 ng of a DNA sample. The mixtures are placed in a thermal cycler; the PCR products are visualized by electrophoresis in 1.5% agarose gel, stained with ethidium bromide, and examined under U.V. illumination. According to the protocol, mixed infections of different genotypes and samples negative for the vacA vaccine are excluded from the analysis.

For molecular diagnostics, a biopsy sample is taken from the antrum of the stomach during gastric endoscopy before starting eradication therapy. During the control of healing, biopsy material is taken 4 weeks after completion of the course of anti-helicobacter therapy from the stomach body. The biopsy is placed in a sterile dry test tube (Eppendorf) and immediately delivered to the laboratory. The biopsy material will likely be frozen at a temperature of -200C for more long-term savings. If patients have gastroduodenal pathology in combination with gingivitis and periodontosis, it is likely to study biopsy material from gums, plaque smears, and saliva. The resulting tissue samples are placed in a sterile dry test tube and delivered to the laboratory for PCR diagnostics. Identifying H. pylori in stool samples using the PCR diagnostic method demonstrated high sensitivity and specificity figures - 83.8% and 98.4%, respectively [1]. False positive results in treated patients can be attributed to the persistence of coccoid forms of H. pylori, which, as a rule, begin to decrease and disappear entirely at 8-12 weeks. The molecular method generally allows the detection and differentiating of strains of the H. pylori bacterium, for example, CagA and VacA. PCR reveals peculiar mutations that lead to drug resistance, which allows resistance to macrolides and fluoroquinolones to be detected before starting therapy. The method allows the bacterium to be shown in any form, amount, and coccal. The PCR primer is obtained from the nucleotide sequence of the urease A gene or in H. pylori. These primers are specific to all H. strains. Pylori is not found in other forms of microbes, so PCR is prepared in a highly specific way. Apart from this, PCR is a more susceptible way to compare with other methods of diagnosing H. pylori infection and allows the detection of 1.47 pg of DNA, among other things. The affectation and specificity of this method formalize 95% and 100% following this [5].

The main disadvantages of PCR are that the technique is expensive and requires many skills and experience. False positive results can also be detected in PCR due to the detection of DNA fragments of already dead bacteria [5,11]. The disadvantage is the possibility of contamination of samples, but this can be avoided by using biosafety standards at all process stages. It should also be noted that the number of bacteria decreases after antibacterial therapy and microbiological and histological tests can give false negative results. However, PCR is a sensitive method that makes it possible to amplify even a small amount of bacterial DNA in various biological samples [12].

It must be remembered that the most reliable method for detecting Helicobacter pylori is PCR diagnostics. In addition to revealing the molecular and genetic composition, it creates an environment for further study and prediction of the disease.

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