Diagnostic Evaluation of Brushing Cytology in the Diagnosis of Helicobacter Pylori Induced Gastritis

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Abstract— The helicobacter pylori is the factor of most frequent infection all around the world and it has been recognized as one of the main reasons of peptic ulcer disease and an important dangerous factor for gastric cancer. There are numerous reliable diagnosis methods for identifying of helicobacter pylori during endoscopy of upper digestive system; biopsy urease is the cheapest and histology is the most expensive tests while brushing cytology is faster and cheaper than histology. In the current study, we clinically evaluate these three tests (biopsy urease, histology and brushing cytology tests). The slides of brushing cytology pertaining to 109 patients who were complained from dyspepsia and were candidate for endoscopy of upper digestive system were collected. The biopsy urease and histology, with tests with H&E coloration, tests were performed for each patient. The presence of helicobacter pylori in each patient was demonstrated by adjustment of at least two tests. Among 100 patients, 78 persons were diagnosed positively by brushing cytology from helicobacter pylori viewpoint. Using the positive and negative answers of two other tests as golden standard, true positive in 48 cases, true negative in 18 cases, liar positive in 7 cases and liar negative in 2 cases were recorded. Therefore, the sensitivity and trait of brushing cytology was obtained as 96 % and 72 %, respectively. The sensitivity of brushing cytology (96 %) was higher than biopsy urease test (77.4 %) and the trait of biopsy urease test (100 %) was higher than histology test (90 %). The brushing cytology is a reliable, cheap and simple method in fast diagnosis of helicobacter pylori infection. When there is not need to extra information about the severity of mucous hurt or presence of atypical cells, the suitable cost – efficiency strategy, rather than histology test, may be consisted of taking a biopsy sample from antrum for the biopsy urease test and preparing the slide of brushing cytology which only will be colored and evaluated if the result of biopsy urease test become negative.

Index Terms— Helicobacter, Gastric cancer, Endoscopy, Biopsyurease test, Histology test, Brushing cytology test, Atypical cells

1 INTRODUCTION

Helicobacter pylori is the most frequent reason of infection all around the world and it is considered as the reason of many gastric – intestinal system diseases [1-8]. Helicobacter pylori may be caused to gastritis, ulcer and duodenal ulcer and, rarely, caused to stomach lymphoma and gastric cancer [9, 10]. In addition, a number of non-digestive diseases such as systemic autoimmune diseases, atherosclerosis, hives and migraine also correlated to helicobacter pylori [11, 12].

Although the infection of helicobacter pylori is often without any sign, the bacteria is accepted, in recent years, as an important factor in ulcers and duodenal ulcers and about 90 % of adults in developing countries and about 30 % of adults in advanced countries are subjected to this infection [13].

Culture and histology with silver coloration are mentioned as the best methods in diagnosis of helicobacter pylori in different references [14]. Recently, numerous methods for diagnosis of helicobacter pylori are under investigation and the brushing cytology is considered as a fast and simple method in diagnosis of helicobacter pylori [15-18].

In the current study, it aims to compare the brushing cytology with various customary methods in diagnosis of helicobacter pylori in order to diagnostically evaluate these methods [19, 20].

The helicobacter pylori infection is a global infection which affects people with weak economical and social position in undeveloping countries [21].

The helicobacter pylori are a microaerophilic bacillus, warm negative and spiral bacteria which its dimension is about 0.3 × 5.5 micrometer [22]. This bacterium is colonized in stomach of human and most types of animals and is of an important role in pathogenesis of gastritis, ulcer and gastric cancer [23]. The first time of reporting a relationship between this bacteria and human disease was in 1979 to 1983 that Marshal and Warren were successfully cultured this bacterium [24, 25]. Although the relationship between helicobacter pylori with gastritis and ulcer was discovered in 1979 by Robin Warren and the bacterium was successfully cultured in 1983 by Barry Marshall, the principal history of the bacterium is backed to the silent time of searching for helicobacter pylori in 19th century in about 20 countries [26].

For example, in 1875, the bacterium was separated from surface and margin of ulcer by Bottcher and Letulle which this is the first hypothesis of relating a bacterium to the ulcer as a primary factor [27].

In 1889, a spiral and springy type bacterium was found and reported in gastric aspirate by Jaworski. In 1893, the presence of spirochaete in mucus of dog's stomach (in filtration of gastric glands, which was found in cytoplasm and vacuoles of partial cells) was reported by Bizzozere and hence, this microorganism was named as Bizzozerian helicobacter in 1996 [28-

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30]. In 1896, Salomon was successfully infected the laboratory mouse by Bizzozerian helicobacter [31]. In 1906, Krinitz was reported the presence of spirochaetes in gastric aspiration of a gastric cancer patient. In 1921, Edkins (the pathfinder of gastrin in 1905) was described the physiology of helicobacter felis in cat [32]. In 1938, Doenges was reported the relationship between spirochaetes and gastritis in macaques monkey and human [33]. In 1940, Freedberg and Barron were confirmed that this microorganism has a non-etiologic role in digestive system diseases of human [34]. In 1940, a hypothesis was presented by Gorham which expressed an acidophilic bacterium is the main reason of ulcer [35]. In 1979, Warren was identified the campylobacter pylori as a primary factor in gastritis of human [36]. In 1983, Marshall was isolated and cultured the campylobacter [37]. During 1985 to 1987, the feeding of campylobacter, on the volunteer persons, as the reason of gastritis in human was studied and described [38]. In 1994, National Institute of Health (NIH), based on the performed studies, was concluded that there is a risk in relation to helicobacter pylori and ulcer [39]. In that year, International Agency of Research on Cancers (IARC) which is a member of World Health Organization, was categorized helicobacter pylori as a carcinogen material of group I [40] and in 1997, the helicobacter pylori genome was identified and separated by Genomic Research Organization [41]. Although the most of helicobacter pylori infection is of no sign, the bacterium is accepted as an important factor in ulcer and duodenal ulcer in recent years [42-55]. Even though the helicobacter pylori infection is medicable, about 90 % of inhabitants in developing countries and 30 % of inhabitants in advance countries, like United States, are infected by the bacterium [56-67]. Since the treating of the helicobacter pylori infection is complex and need to use of at least two types and sometimes three types of different antibiotics simultaneously and also to preventing from discharge of gastric acid in order to efficiently act in deletion of bacterium during treatment time [68-73], it is impossible that half of the world population take antibiotics due to this infection [74-79]. Therefore, prevention methods and possibility of separation of helicobacter pylori from pathogens and non-pathogens types can be very important [80-85].

2 HELICOBACTER PYLORI GASTRITIS

Chronic gastritis is one of the most prevalent chronic diseases of human and it is currently identified that the result is exclusive and non-exclusive responses of gastric mucosa against the helicobacter pylori infection (HP). The HP infection is mostly accompanied by duodenum ulcer and ulcer and is approximately accompanied by all cases of MALT gastric lymphoma. In some special places of the world, there are numerous cases of meta-plastic atrophic gastritis patients which are known preparatory factor of gastric carcinoma. In addition, some out of gastric cases such as systemic autoimmune diseases, atherosclerosis, hives and migraine also are related to HP infection.

2.1 Clinical Signs

The initial phases of HP infection show as an acute mucosa boil response which its clinical signs may be including epigastric pain, nausea and vomiting. Such signs are not frequent and usually are short time. Since the patients rarely tested with endoscopy in initial steps of the HP infection, the information about clinical signs of the disease is limited.

In patients of non complicated HP chronic gastritis, the frequency of dyspepsia is not possibly more than sound people and among the patients of non-ulcer dyspepsia, the frequency of infected and non-infected persons is similar. In addition, it was not shown that treatment of HP in patients of non-ulcer dyspepsia lead to improvement of dyspepsia signs. Even with the presence of an unclear relationship between HP and dyspepsia, it is estimated that HP infection is the responsible for about 5 % of gastric complaints in the society and the patients of HP induced gastritis are subjected to the increasing risk of duodenal ulcer and ulcer and gastric cancer and lymphoma.

2.2 Epidemiology

In many developing countries, the frequency of HP in adults is about 90 % which is along with a high percentage of infected children that shows the age of HP risk is reduced to the initial steps of the lifetime in these countries. At the other hand, in industrialized countries (Western Europe, United States, Canada and Australia) this infection affect people in posterior steps of lifetime which lead to low percentage of children infected by HP (in 2000, in schools of Sweden and Denmark the percentage is lower than 1 %). Therefore, a low percentage of adults also are infected by HP (about 30 % to the age of 50). Although the mechanisms of disease transmission are not clearly known, it was seen that the improving of social-economical conditions lead to decrease in frequency of HP as obviously shown in Finland, Sweden and Japan. Numerous people take Amoxicillin which is not used with other drugs and hence, may be improved about 10 % of HP infections. In addition, it may be possible that other Antibiotics also used daily to treat respiratory infections and other infectious diseases. Among the patients of HP which were under such remedial actions, a low percentage, but considerable, of patients had treated. Finally, the anti HP remediation of single patients possibly reduced the transmission of it in the society and ultimately, the decrease in frequency of HP may be related to decrease in frequency of infection in the population.

2.3 Endoscopy Signs

The chronic HP gastritis has not any individual pattern. Depending on the step or propagation of gastritis, some signs such as hyperaemia, erosion, ulcer, hypertrophy and atrophy may be presented in various compositions with each other in their vicinity or specious regions. None of these types are useful for prediction of presence or lack of HP, as yet. Therefore, the histopathologic analysis is necessary for diagnosis of HP gastritis.

2.4 Histopathology

The HP organisms are infested in gastric mucosa and they are snapped to the surface of mucosa cells. The bacteria are presented in inter-cells spaces, especially in patients that received long time anti-discharge remedy with anchors of proton pump (in the canaliculies of partial cells). The problem of seeing bacteria in the last described position, before developing of poly-

valent coloration techniques, may be misled to that HP is inhabited in the surface of stomach.

The antrum, oxyntic and cardia mucosa are equally sensitive to infection and similarly are colonized. In patients of widespread antrum intestinal metaplasia, infection is limited to non-metaplastic body of stomach which this case is frequent in patients that take anchors of proton pump.

The discharge of gastric epithelium by polymorphonuclear neutrophils is the most exclusive aspect of infected gastric mucosa by HP. Neutrophils are usually more presented in antrum and cardia than body of stomach. HP is rarely presented in body of stomach or even is absent in spite of observable colonization of bacteria. The propagation of gastritis characterizes the more gastritis in antrum which is the most frequent type of gastritis in western countries. Neutrophils usually are the only gastritis cells that infiltrated gastric epithelium in the presence of HP infection, but they are always mixed with lymphocytes, plasma cells and varied amount of eosinophils in lamina propria. Gastritis is tending to be most severe in surface regions of lamina propria; in addition the term "surficial gatrit" is still used as synonym of non-atrophic gastritis.

The lymphoid follicles are always found in infected stomachs and their presence is a reliable indication of active or recently remediated HP gastritis. They mostly aggregated in incisura angularis regions while they rarely found in proximal of large curvature of stomach.

2.5 The Specific Virulence Factors of Disease

Since the identification of HP toxins (cagA and Vaca) which are related to the special types of gastric mucosa damages, continuous efforts are performed to relate them and other virulence factors with exclusive sign or conclusions of HP gastritis. Although some of these factors may be affective on the severity of gastritis or conclusions of gastritis, there is no any clinical usage for tests that identify the potential pathogenesis diagnosis of HP in a patient.

The most well-known virulence factor which has been studied is the gene product related to cytotoxin (cag A). Some signs in the patients of HP with functional cag is seen such as increased IL-8 mucosa surfaces, considerable discharge of neutrophils into the gastric mucosa and increase in risk of developing of symbolic conclusions (e.g., peptic ulcer or gastric cancer). However, the relationship between the presence of cag and its consequence in different geographical regions, especially in east of Asia which more than 90 % of cag is reported in this region, is not compatible. Western countries have more percentage of cases of HP stubs with no cag than Asian countries. This case can describe the possibility of increasing in symbolic consequence. However, the presence of cag has not any value in predicting of clinical signs at now or future for patients.

Other well-known pathogenesis factors are listed below:
IceA, a restrictive enzyme for bacteria which there is not any biologic or epidemiologic document for identifying it as a virulence factor in HP disease and VacA that is divided to a S1 genotype (presumably related to duodenum disease) and a S2 genotype with low ulcergenic potential. A set of studies including about 1500 isolated from United States, Europe and Asia have shown that VacA genotype is not useful for prediction of gastritis degree, signs or response to remediation.

The connected adhesin to blood group antigen (BabA) is an external membranous protein which seems it is effective in connection of HP to Louis b blood group antigen (Leb) on the epithelium gastric cells. A few studies suggested that infection by mouse has babA2, cagA+ and vacAS1 genes (triple positive stubs) may be related to duodenum ulcer but a multi-national larger study was not confirmed this relationship.

3 DIAGNOSIS

Diagnosis of HP gastritis is based on the identifying of HP in gastric mucosa. When the searching for HP is only based on the availability of gastric biopsy samples (to use in histopathology, RUT or culture), only the patients that need to endoscopy will be tested. Developing of inoffensive tests, which their accuracies are continuously increasing, allow to accurate diagnosis of HP gastritis based on indirect methods (i.e. without any need to identification or organism culture). In addition, availability and general acceptability of new, simple and cheap tests lead to developing of indications of HP gastritis diagnosis to a larger group of patients and conditions, even including patients that arbitrarily confer to medical clinics.

Our knowledge about the factors that may be possibly affects the diagnosis results and its accuracy is considerably developed. The prevalence of a condition in an under investigation population affects both positive and negative news value of tests, even if sensitivity and trait of test are independent variables. Therefore, the medic should familiar not only with applicable parameters of applied tests, but also should consider the potential deficiencies of sample preparation and HP prevalence in under evaluation population.

3.1 Offensive Tests

The histopathologic test of gastric biopsy samples: the helicobacter types can be identified by various methods in colored histologic samples of gastric biopsy. H&E method is a suitable choice for coloration of exclusive diagnosis of HP. The special reliable colorations including warthin-Starry, Steiner silver coloration, giemsa, Diff-Quick, Gimenez coloration and triple coloration, which is a regulated composition of Steiner, H&E and Alcian blue colorations, allow the simultaneous monitoring of gastritis aspects including intestinal metaplasia and bacteria which is used by some laboratories to routine clinical diagnosis.

In-situ hybridization and PCR: The in-situ hybridization may be used to HP diagnosis in paraffinic sections but high cost and technical problems limited this technique to research labs. PCR is also considered as a research method for diagnosis of HP infection since it needs to an advanced molecule biologic lab and availability of suitable and expensive primers. Smear, brushing and Touch preparation: the smears of gastric mucosa and epithelial exfoliated cells allow the diagnosis of bacteria

during a few minutes by endoscopy, usually, by a warm coloration.

Culture of bacteria: The helicobacter pylori can cultured, in best form, in a microaerophilic and wet atmosphere on the horse or sheep blood and antibiotic to weakening of other bacteria, as the culture media. However, the culturing of helicobacter pylori is technically more tougher than what that performed in usual clinical microbiology labs.

The fast urease test: This method tested the high amount of urease in helicobacter pylori. A part of gastric mucosa placed into the agar with various concentrations of urea. The produced urease by helicobacter pylori hydrolyzed urea and released ammonium and a suitable indicator (e.g., red phenol) changed the color with increasing pH. In the first commercial RUT (CLO test), the color of a yellow gel capsule which was placed into the sample was changed to red during a few minutes which this was dependent to the amount of presented bacteria. There are various available commercial RUT. Both the sensitivity and trait are very higher than histopathologic test and in most of cases, it reports as about 100 %.

3.2 Inoffensive Tests

The development of new techniques minimizes the problem of crossing reaction which is presented in the first generation of serologic tests. The high number of studies to find the favorite diagnosis test of helicobacter pylori infection provide valuable information about the response of immune system to this organism. One of these information is the selecting of helicobacter pylori types as antigen sources for trait and sensitivity of vital test.

The simplified in-office immuno enzyme tests: There are some in-office tools to fast diagnosis of anti helicobacter pylori IgG. The most of them are one-time kits which shown the answer yes/no during a few minutes after placing a drop of serum. Although some of these tests are accurate, the results are usually of lower sensitivity and trait than necessary standard (90 %). The antibodies (often from IgG) against helicobacter pylori are identified in saliva and urine of patients. The sensitivity and trait of urine tests are acceptable in some studies, especially in Japan.

Evaluation of stool antigen: The immunoassay enzyme (HPSA) that identifies the antigen of helicobacter pylori in stool (hence, provides some information about the presence of current infection) is used for diagnosis of helicobacter pylori infection and monitoring of response to remediation. This test is similar to immunosorbent assay connected to enzyme which uses polyclonal antibody against helicobacter pylori connected to micro wells. A study performed by some big European centers was shown hopeful results about this test. The respiratory urea test: this test is among the most important and innovative methods to diagnosis of helicobacter pylori infection. This test is based on the ability of helicobacter pylori in producing high amount of urease. Drinking of a solution containing urea by an infected person leads to fast production of ammonium and carbon dioxide which is quickly observed in breath of person. If the urea is signed with radioactive isotope 14C or non-radioactive isotope 13C, the carbon dioxide in breath also is signed. Hence, it is measureable with a suitable diagnosis method. When the urea signed with 14C is used, the general method is drinking a solution or eating a capsule containing 0.5 to 10 microcurie (μci) of urea signed with isotope. When the urea signed with 13C is used, the patient should drink a 125 gr solution of 99.9 % signed urea and then should eat a meal to increase the time of presence of solution in stomach. After a period of time, the patient should blowing into a balloon which immediately blocked and sent to a laboratory to identify the carbon dioxide signed with isotope. Both tests are standardized and are confirmed by regulated agencies in Europe and North America. Opposite to the serologic tests, the respiratory urea test is infinitely sensitive and trait and identifies the active current infection (not observations from past infections). They are selected tests for some groups of population including children, pregnant women and patients which are not able to test with endoscopy.

4 Conclusion

At now, the only treatment indications which are generally accepted and agreed are duodenum ulcer, helicobacter pylori induced ulcer and MALT lymphoma of low grade initial cell. Helicobacter pylori should be deleted in patients of ulcer which presence of ulcer is confirmed, whether ulcer is active or inactive at now. Most of clinical trials have not presented persuasive data in support of advantages of helicobacter pylori infection deletion in non-ulceric dyspepsia and there is not any controlled study which shows that deletion of helicobacter pylori from population would resulted in decreasing of gastric cancer. Due to ethical and logical reasons, it is not possible that such trials will perform to satisfy those which requested suspicious and undetermined observations. The most important question is that if we should expect to more data or act based on the current information. Today, there are numerous epidemiologic and biologic observations which helicobacter pylori induced gastritis is not the only reason of atrophy and intestinal metaplasia frequency. However, it is always the necessary and imperative basis for such diseases. Therefore, we should be able to preventing gastric cancer. In laboratory, helicobacter pylori is sensitive to wide range of antibiotics but single-drug remediation was not successful in human which is probably due to releasing of insufficient antibiotic to the place of colonization. As a result, various multi-drug regimes have been developed that their most successful is a triple and quadruple regime which its rate of helicobacter pylori deletion is more than 90 % in many trials and is more than 75 % in clinical experiences. The most prevalent 7 and 14 days drug regime consists of anchors of proton pump and 2 or 3 antimicrobial factors.

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