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RESEARCH ARTICLE

DIAGNOSTIC UTILITY OF THE RAPID UREASE TEST FOR HELICOBACTER PYLORI IN GASTRIC JUICE SAMPLES

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Abstract

Objective: The purpose of this study is to compare the diagnostic performance of the rapid urease test for *H. pylori* in endoscopic samples of gastric juice compared with the same test in gastric mucosa. Materials and methods: Cross-sectional, comparative and prospective study with study of concordance of diagnostic tests, carried out with patients referred to a medical center in Bogotá DC, Colombia for the performance of an esophagogastroduodenoscopy. We included 130 patients older than 18 years, without antibiotic treatment or inhibitors of gastric secretion, or with any type of immunodeficiency, or cancer. Were processed in the sensibacterpyroli test device a sample of gastric antrum for Rapid Ureasa Test RUT in mucosa and for the sample of juice 5 mL was taken with suctionator 7A-23B pulmomed (USA) R, to compare results. Results: In this study the infection by *Helicobacter pylori* was detected by biopsy sample in 40.8% of the patients that corresponded to 53 patients, among (59.2%) 77 patients with negative values. The prevalence of H. pylori infection in this study was 40.8%. It was calculated of VPP and NPV: 95% and NPV: 89.3%. Conclusions: rapid urease test in gastric juice is a fast and economic test, with very good sensitivity and specificity, is also very useful to detect *H. pylori* infection.

Introduction:-

Helicobacter pylori (*H.pylori*) is a bacterium involved in the pathogenesis of different human diseases such as atrophic gastritis, gastric and duodenal ulcers, gastric neoplasms such as adenocarcinomas, MALT lymphomas, and nutritional deficiencies of iron and vitamin B12 (1). *H.pylori* has a low tolerance to oxygen and produces the enzyme urease that hydrolyzes urea in body fluids to CO2 and ammonia, alkalizing their environment. This characteristic allows it to adapt to a highly acidic gastric environment and trigger the host's immune response, the end result of which is chronic inflammation of the gastric epithelium (2). Three invasive diagnostic methods are available to detect *H.pylori*infection; rapid urease test, histopathology, and bacterial culture of mucosal samples. Noninvasive options for diagnosis include serum or urine IgG class antibodies (SHPAb, UHPAb), the 13C breath test (UBT), and stool antigen testing (SHPAg) (3). The diagnostic performance of these tests varies in relation to the prevalence of infection in the populations studied, in addition to their availability and economic costs being variable. All tests used commercially have proven to be accurate and reliable, but a single test with the exception of selective culture (considered the gold standard) is not sufficient to make a definitive diagnosis. However, in clinical practice

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the diagnosis of infection is based on the positivity of a single test, therefore choosing the best one in the clinical setting is essential, before deciding whether or not the patient requires antibiotic eradication treatment (4). (5)

The urease test (RUT) is an indirect test that detects the presence of *H.pylori* based on the presence of the enzyme urease in the gastric mucosa. The test requires a sample of gastric mucus or mucus which is placed in a device containing urea and a method that detects the products of urea hydrolysis such as CO2 or ammonia. The phenol red dye is used as an indicator of the change in pH, the change in color of RUT from yellow to pink or red indicates an increase in pH and the presence of ammonia. The "homemade" RUTs that contain small amounts of substrate and pH indicator, have proven to be very useful with low economic costs (6) (7). The present work aims to compare the diagnostic performance of the rapid urease test for *H.pylori* in endoscopic samples of gastric juice compared to the same test in gastric mucosa.

Materials and Methods:

Cross-sectional, comparative and prospective study with a concordance study of diagnostic tests, carried out between January 2 and July 30, 2017, with patients referred to the EndocentroLtda medical center in Bogotá DC, Colombia for an esophagogastroduodenoscopy. The patients agreed to participate voluntarily and signed an informed consent. The study was approved by the research committee of the institution. Patients older than 18 years, who had not received antibiotic therapy up to one year before the examination, drugs that inhibit gastric secretion up to twelve weeks before endoscopy, who received immunosuppressive or immunomodulatory drugs, who had cancer in any organ, or with some type of immunodeficiency.

For the examination, Olympus videoendoscopes, Willson Cook biopsy forceps were used, for RUT in mucosa a sample of the gastric antrum was taken which was introduced into a 5 ml micropipette with the Sensibacter test, a 5 ml sample of gastric juice was aspirated in a sterile container used to trap polyps, 2 ml of this amount was placed on the Sensibacter device. The samples that turned from yellow to red according to the manufacturer's recommendation, at 24 hours, were considered positive. The population sample consisted of 130 patients calculated in the EPIDAT 3.0 program with an expected proportion of 0.5% and a precision of 1%. The data collection was recorded in an Excel table once the tests had been performed, for the statistical analysis the SPSS version 19.0 program was used. For quantitative variables, measures of central tendency were made such as mean, median and mode, ranges, minimum value and maximum value, for qualitative variables they were described in terms of absolute values and percentages. In the statistical package of social sciences SPSS, cross tests were carried out, subsequently sensitivity and specificity tests, with 95% CI, the PPV and NPV were calculated by means of the following formulas:

$$PPV = \frac{P(\text{disease})\, p(+ / \text{disease})}{P(\text{disease})\, p(+ / \text{disease}) + p(\text{healthy})\, p(+ / \text{healthy})} \times 100\% =$$

$$NPV = \frac{P(\text{healthy})\, p(- / \text{healthy})}{P(\text{healthy})\, p(- / \text{healthy}) + p(\text{disease})\, p(´ / \text{disease})} \times 100\% =$$

Cronbach's indices were analyzed to determine how reliable the gastric juice test is compared to that taken by biopsy, and finally the concordance test between samples with Cohen's Kappa index was calculated. Therefore, the null hypothesis was raised: There is no concordance between the results of gastric biopsy and gastric juice for the diagnosis of *Helicobacter pylori* infection in adults. As an alternative hypothesis: there is a concordance between the results obtained from samples from gastric biopsy and gastric juice for the diagnosis of *H.pylori* infection.

Results:-

130 patients were included, of which 99 were women (75.6%) and 31 men (23.7%), with a mode of 52 years and a mean of 46.62, SD 14.19, ages between 18 up to 81 years of age. The two types of samples were taken from all patients, the results obtained were blinded between observers. In this study,*Helicobacter pylori* infection was detected by biopsy sample in 40.8% of the patients, corresponding to 53 patients, among (59.2%) 77 patients with negative values. The prevalence of *H.Pylori* infection in this study was 40.8%

Table 1:-

TABLA 1:-Comparasion between the results of the two types of samples for detection of *H.pylori* of negatives		
Type of sample	**Number of positives**	**Number of negatives**
Biopsy	**53 (40.8%)**	**77(59.2%)**
Gastricjuice	**41 (31.5%)**	**89(68.5%)**

The sensitivity and specificity of the samples taken by gastric juice were 90.2% and 82.0%. The data obtained from the biopsy sample was considered as a reference. For this, the cross-tests were performed versus the gastric juice test in the SPSS program. PPV and NPV were calculated according to Bayes' theorem, PPV was obtained: 95% AND NPV: 89.3%

VVP = represents the 95% probability if the result is positive.
NPV = 89.3% of a patient is healthy given that the result has been negative

The Cronbach's alpha test was used as a reliability statistic, whose index is above 0.8, which indicates that the gastric juice test has good reliability with respect to the biopsy sample. For a 95% confidence interval there was a correlation between the tests of 0.682 and mean measures of 0.811 associated with Cronbach's alpha. The concordances between the results between the two samples were compared by means of the Kappa coefficient that gave 0.670, which indicated that the concordance between the samples was satisfactory, according to the Cohen scale (0.61-0.80 good concordance). Therefore, the results obtained with this result in this sample are valid and reliable.

Discussion:-

The urease complex, in addition to having a direct cytotoxic effect on gastric cells, the ammonium produced interferes with the normal diffusion of hydrogen in the gastric mucosa, and induces inflammation with the recruitment of leukocytes, the production of pro-inflammatory cytokines, and the production of reactive oxygen metabolites. Furthermore, urease has been observed to induce chemotaxis and modulation of the immune system (7) (8). For a positive RUT, more than one hundred thousand *H.pylori* are required in the gastric biopsy, this is generally not a problem since the density of the infection in the human mucosa exceeds this limit (9). The reported sensitivity and specificity of various commercial and / or "homemade" RUT ranges from 80% to 100% and 97% to 99% respectively (10). In patients with non-atrophic gastritis or duodenal ulcers, the presence of *H.pylori* in biopsies of the antrum is large and the diagnostic yield of the RUT is greater than 85%, however in patients with atrophic gastritis and intestinal metaplasia, the density of the infection is low and the results of the RUT can be disappointing (11). As the distribution of *H.pylori* in the gastric mucosa is focal, when a single sample is taken for the RUT, false negative results can occur with important clinical consequences, in contrast, gastric juice contains mucus, desquamated epithelial cells and bacteria from all over the stomach and may be an appropriate means of detecting infection (12). The rapid urease test (RUT) was performed on gastric juice samples and biopsies from 130 individuals, most of them women. The method used for gastric juice collection was fast, well tolerated and with an excellent concentration of bacteria; proving to be suitable for the purpose of the work.

It is known that the RUT test in gastric biopsies has a sensitivity greater than 90%, and a high specificity that reaches 100%. In this study, it was found that the sensitivity of RUT in gastric juice was 90.2% and specificity 89.3%, findings similar to those found (11).

A prevalence of infection of 40.8% was found in the population studied; observing a meaningactive decrease in the prevalence of Helicobacter infection, compared to previous reports where the prevalence was 36.4%, suggesting that the rate of disease acquisition has decreased and that sanitary conditions in general have improved (13). A meta-analysis of 2017 shows an important variation between regions, countries and continents, they report a prevalence of 63.4% for South America, the highest figure is in Africa with 79.1% and the lowest Oceania with 24, 4%. They estimate that there are approximately 4.4 billion people in the world positive for *H. pylori* (14). The detection of RUT in gastric juice is a good choice for several reasons such as speed, lower economic cost, and comparable results with traditional diagnostic methods. Furthermore, RUT in gastric juice is useful in histologically negative patients who have histopathological changes associated with H pylori infection (12). Given the significant volume of patients who demand diagnostic tests by medical order, it is imperative to find a method that can be performed in the endoscopy room itself and that also contributes to reducing expenses not reimbursed by the health system.

Endoscopic biopsy requires the use of a special forceps reusable or not, with a unit cost that greatly affects the cost benefit of the procedure and also increases the economic value by requiring transportation, material processing, reading by pathologist and also increases the burden endoscopy services generating cost overruns of up to 80%. Although non-invasive diagnostic tests are available and their use is endorsed by the clinical practice guidelines of the Colombian Association of Gastroenterology, their use has not spread and therefore reason for its usefulness from the cost-effectiveness point of view, which seems to be important, has not been quantified in our environment. The RUT can offer an alternative that reduces costs and given its good performance found in this study. The concordance index greater than 60%, presented for both the positive and negative results for RUT in gastric juice, is a real low-cost alternative that can impact the diagnosis of *H. pylori* infection. In conclusion, the rapid urease test is a fast, cheap test with very good sensitivity and specificity, it is also very useful to detect the urease enzyme produced by the Helicobacter genus in gastric mucosa samples such as biopsies and gastric juice.

In this study, the agreement or accuracy between the results obtained with the two types of samples was 0.670, indicating good agreement between the samples.

Although the biopsy sample presented a greater number of positive results 50 (43.8%), it is surpassed by the number of negative results within the same test 77 (59.2%) in this study; however, a directly proportional relationship is observed between both tests with respect to the frequency of positive and negative data between tests (see table 1).

It would be of interest for future studies to compare the rapid gastric juice test with the bacterial culture test in a similar population.

It is important to note that the diagnostic performance of the test is related to the prevalence of the disease, which for this study was 40.8%, which is why it is related to the presence of a considerable number of negative values in the results of biopsy samples. (see table 1) and supported by. Ricci C, Holton J, Vaira D in 2007. (15) (16)

Conclusion:-

The authors suggest that invasive diagnostic methods to define *H. pylori* infection, such as histology and culture, must have specialized staff to perform these tests, in institutes with the necessary equipment to perform endoscopies, the use of the rapid urease test can be implemented starting from alternative samples such as gastric juice.The rapid gastric juice test can be suggested for screening patients, in populations in which the need for a diagnosis to define *H pylori* infection is required, as additional support to the health professional.

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