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DIAGNOSIS OF Helicobacter pylori: COMPARISON OF AN UREASE TEST, HISTOLOGICAL VISUALIZATION OF CURVED BACTERIA AND CULTURE

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SUMMARY

Helicobacter pylori was investigated in 189 patients for culture, microscopic visualization of campylobacter-like organisms (CLO) and a ten minute urease test. In 136 (72%) the bacteria was isolated, and in 98 of them CLO were histologically detected. Specificity, sensitivity, positive and negative predictive values of microscopic visualization of CLO were: 0.77, 0.73, 0.97 and 0.51, respectively; 98 culture-positive patients were urease test positive. Specificity, sensitivity, positive and negative predictive values of the urease test were: 0.83, 0.72, 0.92 and 0.54, respectively. Comparing the urease test with culture of **H. pylori** combined with microscopic visualization of CLO, its specificity, sensitivity, positive and negative predictive values were: 0.95, 0.71, 0.98 and 0.48, respectively. Probably, these values are not real, since bacteria different from **H. pylori** could be misclassified as CLO.

KEY WORDS: Campylobacter pylori; Helicobacter pylori; Diagnostic; Technical assay; Campylobacter-like organisms; Urease test.

INTRODUCTION

Helicobacter pylori, previously called **Campylobacter pylori**[2], is a curved Gram negative bacterium strongly associated with active non-specific gastritis and peptic ulcer. This bacterium can be identified in gastric biopsies by culture and by direct observation of histologic sections stained with hematoxilin-eosin (HE), Giemsa, Giménez, and silver-stains[3, 12]. Both techniques, cultural isolation and observation, are time consuming. Fortunately, this microorganism presents a strong urease reaction due to a performed enzyme[6, 9]. This characteristic has been used for its rapid detection by direct inoculation of the gastric tissue in urea solution[1, 4-5, 7, 9-12].

The aim of this study is to compare an urease test described for RUIZ **et al**[10] with the microscopic observation of Campylobacter-like Organisms (CLO) and with the isolation of **C. pylori**.

MATERIALS AND METHODS

Biopsies from gastric antrum were taken from 189 patients attending at the Endoscopic Service of the Hospital México, San José, Costa Rica. The tissues were evaluated by culture, histological examination, and urease test. For culturing, the tissue was rubbed across the surface of a blood agar plate and incubated under mi-

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croaerophilic conditions for five days at 37°C. The microorganisms isolated were identified as **Campylobacter pylori** on the basis of their colony morphology, microscopic morphology with the Gram stain and positive reactions for urea, oxidase, and catalase. After using it for the inoculation of the blood agar plate, the same gastric tissue specimen was placed into a tube with 200 μl of 10% urea with 0.2% phenol red. The tube was hand shaken and inspected for the development of a pink colour within the first ten minutes, which was interpreted as positive.

For histologic examination an additional biopsy was fixed in 10% bufferized paraformaldehide and cut sections were stained with HE and Giemsa. Histological analysis, urease test and bacterial cultures were interpreted by independent observers without communication among them. Statistical analysis were done with the Pearson's X^2 and its contingency coefficient factor.

RESULTS

Of the 189 patients, 136 (72%) were found to be **C. pylori** culture positive. Only the biopsies of 186 patients were histologically analysed (3 samples — two culture positive — were excluded because they were inadequate for the histological process). In 98 of the culture positive specimens CLO were histologically visualized (true positive cases). In the remaining 38 culture positive cases, the bacteria were not visualized (false negative cases). On the other hand, there were 15 culture-negative biopsies in which bacilli were observed in the histological analysis (false positive cases).

If we assume that the culture, as diagnostic of **C. pylori**, is an error-free method; its comparison with the microscopic visualization of CLO yields the following estimates: specificity 0.71, sensitivity 0.73; the positive and negative predictive values are 0.87 and 0.51, respectively.

In the case of the urease test, out of the 136 culture positive patients, 98 had positive urease tests (true positive cases) and 38 had negative tests (false negatives). Then, 9 culture-negative cases had urease positive tests (false positives). The remaining 48 patients, culture and urease test negatives, were considered as true negatives. The urease test specificity and sensitivity are 0.83 and 0.72 respectively, with positive and negative predictive values of 0.92 and 0.54, respectively.

On the other hand, if the diagnosis of **H. pylori** had been obtained by a combination of culture and histological visualization of CLO, and such data compared with the urease test, its specificity would have been 0.95 because the false positive results diminish markedly, but the false negatives increased. The sensitivity and predictive positive value had been 0.71 and 0.981, respectively.

DISCUSSION

We consider that the situation, in which the diagnosis of **H. pylori** was done with the culture combined with histological visualization of CLO, is not real; because some culture-negative patients could be colonized by bacteria rather than **H. pylori**, which could be misclassified as CLO.

It is also likely that some specimens, in which CLO were visualized or were urease test positive, were really positives. In any case we assume that the true **Helicobacter** positive titres were those diagnosed by culture. Thus, histological visualization of CLO and the urease test are only presumptive diagnosis tests, which have a good correlation with positive cases (Contigency coeff. 0.38); but, with defficiency in the identification of negative cases. However, their negative results need confirmation by culture.

RESUMEN

Diagnóstico del Helicobacter pylori: **comparación entre la prueba de urease, examen histológico de bacterias curvas y el cultivo.**

Se investigo la presencia de **Helicobacter pylori** en 189 pacientes, mediante cultivo, visualización microscópica de organismos parecidos a Campylobacter (OPC) y una prueba rápida de ureasa. La bacteria se aisló de 136 (72%) casos y en 98 de ellos se observaron OPC. La especificidad, sensibilidad y los valores pedictivos positivo y negativo para la visualización microscópica de OPC fueron: 0.77, 0.73, 0.97 y 0.51 respecti-

vamente. La prueba de ureasa fue positiva en 98 de los pacientes positivos por cultivo. La especificidad, sensibilidad y los valores predictivos positivo y negativo para la prueba de ureasa fueron: 0.83, 0.72, 0.92 y 0.54 respectivamente. Cuando se comparó la prueba de urease con el diagnóstico de **H. pylori** realizado combinando el cultivo con la visualización microscópica de OPC, su especificidad, sensibilidad y los valores predictivos positivo y negativo fueron: 0.95, 0.71, 0.98 y 0.48 respectivamente. Esta última condición no es real por cuanto microscópicamente otras bacterias diferentes de **H. pylori** podrían ser erróneamente clasificadas como OPC.

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