Comparison of different diagnostic methods of *Helicobacter pylori* infection in Sudanese patients

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ABSTRACT

This study aimed at comparing a PCR method of direct detection from biopsy and two other methods: culture and (campylobacter like organism) CLO test for the diagnosis of *H. pylori* in Sudan. A total of 100 biopsies were taken from 100 patients presenting with various gastro-duodenal symptoms after obtaining an informed consent. The biopsies were analyzed using the culture method, CLO test kit and the PCR test using the 23S rRNA gene (Jene bioscience kits). With culture 48% (48 out of 100) were positive, CLO test 53% (53 of 100) were positive, 58 out of 100 (58%) were patients by using PCR. Sensitivity and specificity of culture technique was (78%) and (94%) respectively while PCR showed a sensitivity of 94% and a specificity of 75% and CLO test showed (96% & 86.5%) for sensitivity and specificity when culture was the golden method and (88%, 95%) when PCR was the golden method. The PCR test appears to be the most reliable test for diagnosis of *H. pylori* in Sudan especially where culture is difficult.

Keywords: Helicobacter pylori, biopsies, PCR, CLO test, culture.

INTRODUCTION

*Helicobacter pylori* (*H. pylori*) is the causative agent of gastritis, peptic ulcer, MALT lymphoma and is a risk factor in the development of gastric cancer (1). There are several methods for the diagnosis of *Helicobacter pylori* and can be classed into two broad categories, namely invasive methods that require endoscopy or the minimally or non-invasive methods that do not require endoscopy.

The endoscopy methods include culture, (campylobacter like organism) CLO test, PCR, fluorescence in situ hybridization (FISH), direct gram stain, histology, while the non-invasive methods include serology, urea breath test (UBT) and *Helicobacter pylori* stool antigen test (HbsAg).

Culture is the gold standard for the diagnosis of any microorganism and so where culture cannot be possible histology
and CLO test have been used as the gold standard for the diagnosis of *H. pylori*. UBT is however the known gold standard for the non-invasive detection of *H. pylori*.(2)

In Sudan, currently both gold standards for the non-invasive and invasive detection of *H. pylori* are fraught with several problems such methods are either difficult for isolation of *H. pylori* due to frequent power outages in case of culture or very expensive and not generally affordable or available in case of UBT. This, in addition to the fact that culture could take several days to obtain a result and in developing countries, of which Sudan is one, it is even difficult to obtain a result.

Several assays based on the use of PCR have been developed to detect the presence of *H. pylori* DNA by using several gene targets directly from the biopsies (2–3). The targets of these PCR methods include urease A (*ureA*) gene, *cagA* gene, phosphosamine mutase (*glmM*) gene and 16S rRNA gene (3,4,5). (3,4,5)

The aim of the study was to compare the sensitivity and specificity of PCR methods in *H. pylori* diagnosis with the results with those of culture and CLO test.

**MATERIALS AND METHODS**

This was a descriptive cross-sectional study carried out in Khartoum state (Sudan).

One hundred biopsies were collected from patients attending Alribat teaching Hospital, Alneelain medical center and Asia hospital, with gastric symptoms. The specimens were analyzed using the following methods: culture method, CLO test and PCR.

The total Gastric biopsies genomic DNA was extracted by using (Jena bioscience) Genomic DNA purification from tissue DNA Preparation Kit according to manufacturer's instructions.

PCR amplification methods and oligonucleotide primers derived from a known sequence of the 23S rRNA gene were used:

5’-AGGTTAAGAGGATGCAGTGC-3’ and 5’-CGCATGATATCCCATAGCAGT-3’

The gel was visualized over ultraviolet trans-illuminator, and photographed using gel documentation system, fragments size (about 267 bp at second PCR reaction) were estimated from the distance of migration relative to the positive control.

Culture was done by direct plating from biopsies samples. The media used include an agar base (brain heart infusion agar), selective supplement (Skirrow) and growth supplement (animal plasma).

The inoculated plates were incubated in microaerophilic atmosphere using microaerophilic kits (campylobacter system CN0025A oxoid) at 37°C for 3 days. The isolated bacteria were identified by its colonial morphology, microscopy and biochemical test.

The CLO test was done using the sample according to the kits manufacture’s instructions.

**RESULTS**
A total number of one hundred patients attending Alrebat teaching hospital, alneelain medical center and Asia hospital suffering from GIT problem were enrolled in this study. Of these (54%) were males and (46%) were females.

The age of the patients ranged between 10 to 80 years, the endoscopic result showed that 82% of the patients have gastritis, 13% with duodenitis and only (7%) have gastric ulcer.

_H. pylori_ were isolated from 48 patients (48%) by culture method and from 58 patients (58%) by using PCR method (Figure .2) and from 54 patients (54%) using CLO test. (Figure 1)

A total number of 45 patients gave positive result in both culture and PCR while 3 patients' showed positive result in culture and negative in PCR and 13 patients were positive by PCR and negative by culture. (Table.1). Chi-square shows that there is difference between culture and PCR (P value: .000).

Forty six patients showed positive results in both culture and CLO techniques, 2 patients gave positive result only by culture and negative to CLO test and 7 patients showed positive result by CLO test and negative by the culture technique. (Table.2). Chi-square showed that there is significant difference between culture and CLO test (P value: .000).

The sensitivity and specificity of culture technique was (78%) and (94%) respectively while PCR showed a sensitivity of 94% and a specificity of 75% and CLO test showed (96% & 86.5%) for sensitivity and specificity when culture was the golden method and (88% & 95%) when PCR was considered as the golden method.

Statistical analysis revealed that culture isolated _H. pylori_ was not significantly associated with the gender of patients (P= 0.083). Also, there were no differences (P= 0.848) in culture isolated rates and patients symptoms (epigastric pain (P= 0.848), heartburn (P= 1.333) and reflux (P= 3.000). Statistical analysis also showed that no significant relation between culture isolate and endoscopic findings (gastritis, gastric ulcer and dudenitis) (P=0.645)

Of the total number of 58 PCR positive cases 32 were males and 26 were females, of these 58 epigastric pain was found in all of them (100%), heartburn in 51.7% (30/58), reflux in 51.7% (30/58) and vomiting in 27.6% (16/58).

Statistical analysis showed that PCR positivity to _H. pylori_ was not significantly associated with the gender of patients (P= 0.621). Statistically there was no association between PCR positive and patients symptoms (P= 0.069).
(Figure 1) Shows % positivity of the 3 methods used for *H. pylori* diagnosis

(Figure 2) PCR gel documentation of *helicobacter pylori*

(Table 1) Correlation between culture and PCR

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(Table 2): Correlation between culture and CLO test

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**DISCUSSION**

*H. pylorus* is a gram-negative bacterium that colonizes the human stomach resulting in chronic gastritis. The severity of the inflammation is likely to underlie *H. pylori*-related diseases.

The prevalence of *H. pylori* infection increases with age worldwide. In the present study, this increase pattern was exhibited partly among our Sudanese.

Accurate diagnosis is essential for the effective treatment and management of infections caused by this organism. There are several methods for the diagnosis of *H. pylori* and can be classed into two broad categories, namely invasive methods that require endoscopy or the minimally or non-invasive methods that do not require endoscopy. The endoscopy methods include direct Gram’s stain, culture, CLO
test, PCR, fluorescence in situ hybridization (FISH) and histology; while the non-invasive methods include serology, urea breath test (UBT) and H. pylori stool antigen test.

In the present study culture, PCR and CLO test were used as diagnostic tools. Of these cultures technique detected H. pylori in (48%) of patients, PCR in (58%) and CLO test in (54%).

Culture is the gold standard for the diagnosis of any microorganism, but in the case of H. pylori many drawbacks were noticed including that it is time consuming and difficult to grow due to the usual none abundance of bacteria and the strict conditions required for its growth: like growth supplements and selective requirements. Out of 100 sample inoculated in brain heart agar 48 H. pylori were isolated. This finding is in accordance to the findings of Reza Khashei and his colleagues who found that 80 (34.8%) of the 230 patients studied were harboring H. pylori in the antrum of the stomach (8). Another study showed that the percentage of culture positive specimen was 31.94% (9).

Several assays based on the use of PCR have been developed to detect the presence of H. pylori DNA by using several gene targets directly from the biopsies (10) (11). The targets of these PCR methods included urease A (ureA) gene cag A gene (12), phosphosamine mutase (glmM) gene (13) and 23S rRNA gene.

The advantage of PCR over non molecular methods is that it affords a high degree of sensitivity and specificity, with a detection rate of 10 to 100 H. pylori cells.

The present study was conducted using PCR targeted at the specific 23S rRNA gene for H. pylori. The positive result of (58%) was close to the study done by Sonia Agudo and her colleagues in USA who found (64.1%) prevalence of H. pylori among Spanish patients (15). It is also similar to another study done in China by Zhuoqi and his colleagues who detected H. pylori in (59.5%) of the studied samples. (16). SI Smith and his colleagues detected H. pylori in 35% of their patients using a PCR method in Nigeria (17).

The rapid urease CLO (Campylobacter-like organism test) is commonly used during endoscopy to diagnose the presence of H. pylori. The basis of the test is the ability of H. pylori to secrete the urease enzyme, which catalyzes the conversion of urea to ammonia and bicarbonate. CLO test results were observed in (54%) of the patients in this study. This finding is in accordance to the study done in Kuwait University, where (52%) of the patients had a positive CLO test when they also used a single biopsy as we did. (18). Our result was different from that of Stella I. Smith and his colleagues who found that the percentage of the CLO test was 15/42 (35.71%) (17).

In conclusion the PCR test was found to be the most sensitive method to be used for the routine diagnosis for the presence of helicobacter pylori in patients suspected to be infected by the organism.

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