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Research Article

EVALUATION OF RAPID UREASE TEST IN DIAGNOSIS OF *Helicobacter pylori* AMONG GASTRODUDENITIS AND PEPTIC ULCER Patients IN COMPARISON WITH PCR IN KHARTOUM STATE

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ABSTRACT

Background: Recently *Helicobacter pylori (H. pylori)* has been involved in many serious diseases such as peptic ulcer and ended with gastric cancer, which can be prevented using specific diagnostic test and treatment.

Objective: The aim of this study was to evaluate rapid urease test (RUT) using Polymerase Chain Reaction (PCR) as gold standard method, for the detection of the *H. pylori* in gastric and duodenal biopsy specimens of gastrodudenitis and peptic ulcer, in Khartoum state, Sudan.

Materials and Methods: Fifty patients with gastrodudenitis and peptic ulcer attending Alribat Teaching hospital during period from March to April were enrolled in this study. Two gastric/duodenal biopsies specimens were collected and subjected for RUT and PCR for detection of 16-S gene. Sensitivity, specificity of RUT was calculated using PCR as gold standard.

Results out of 50 specimens tested 26 were positive (52%) and 24 were negative (48%) by using PCR. Out of 26 positive by PCR 22 were positive by RUT and out 24 negative by PCR 21 were negative by RUT .The sensitivity, specificity of RUT were 84.6% and 87.5%, respectively.

Conclusion in Comparison with PCR , RUT show high specificity and sensitivity. So its use will serve as good alternative to PCR particularly in resources limited area.

Key words: Helicobacter pylori, PCR, rapid urease test, Sudan.

INTRODUCTION

Helicobacter pylori are a Gram negative, spiral, flagellated bacterium with the capability of abundant urease production. *H. pylori* bacterium is usually found under the mucus layer in the gastric pits and in close opposition to gastric epithelial cells [(1)].

Since the discovery of *H. pylori* by Warren and Marshall, it has been evidently demonstrated that the organism plays a major role in several upper gastrointestinal diseases which present as dyspepsia [(2)].

Helicobacter pylori infection causes chronic active gastritis in the antrum (antral gastritis), the corpus (corpus gastritis) or in both (pan gastritis). It is a major etiological factor in peptic ulcer disease, gastric carcinoma, and gastric mucosal associated lymphoid tissue (MALT) Lymphoma, Hemorrhage and perforation are the most frequent complications of peptic ulcer disease and are associated with substantial morbidity, mortality and health care costs [(3)]. Peptic ulcer disease can be cured by eradicating *H. pylori* so that complications no longer occur [(1)].

There are various diagnostic tests for *H. pylori* which can be broadly classified into invasive and noninvasive tests [(4)]. Invasive tests utilize endoscopic gastro duodenal biopsy samples for histology, culture, rapid urease test (RUT), polymerase chain reaction and fluorescent in-situ hybridization. The non-invasive tests do not require endoscopy; they include urea breath test, immunoglobulin G, A and M serology, stool antigen test, saliva antibody test and urinary antibody test [(5)].

Materials and methods

This is a descriptive, cross sectional study was performed with fifty gastroduodenitis patients observed by physician during endoscopy with and\or peptic ulcer attending Alribat Teaching hospital Khartoum state, Sudan during the period from March to May 2015 randomly selected

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based on non-probability convenience sampling technique, Patients under antibiotic treatment against *H. pylori* and patients not fully diagnosed with gastroduodenitis were excluded.

Multiple gastric biopsy specimens were taken from the antrum, the corpus and duodenum, one specimen was placed directly into urea broth media and the other sample stored in sterile normal saline and kept at -80 ***°C*** until DNA extraction.

Rapid Urease Test

Christensen's urea broth was prepared as manufacturer instructions, briefly 0.9 gram powder was added to 95ml of de-ionized water, mixed by swirl then sterilized by autoclave (121° C for 15 minutes), after that 2% of urea crystals was prepared and added aseptically to urea broth base, then dispensed aseptically in 0.5ml into sterile tube, and stored at 4° C till used.

And rapid urease test was done by inoculating biopsy samples into prepared media then it was incubated at 37°C, aerobically, for 2-8 hours. Positive result was indicated when color changed to red-pink while no color change indicates negative results.

DNA extraction from biopsy specimens

Extraction was done by using vivantis kit "Vivantis, Malaysia" (catalogue (GF-TD-100) according to manufactories instruction 5-10 mg of frozen tissue was transferred to 1.5 ml micro tube, then 250 µL of tissue lysis buffer and 20 µL of proteinase k solution was added to the sample, mixed by pulsed vortexing (Lab Tech (LVM-202). until homogenous solution was obtained. then 12 µL of lysis enhancer was added and mixed immediately, then incubated at 65 ***°C*** for 3 hours in a shaking water bath (Scott science U.K, model LWB-122D), then 650 µL of tissue genomic DNA binding buffer added and mixed thoroughly by pulsed vortexing until homogenous solution was obtained and incubated for 10 minutes at 65***°C***, 200 µL of absolute ethanol was added and mixed immediately, 650 µL of sample transferred into a column assembled in clean collection tube, centrifuged at 5000 x g (Hettich zentrifugen (EBA 20)) for 1 minute and flow was discarded then column was washed two times with 650 µL of washing buffer and centrifugation for 5000 x g for 1 minute. Column centrifuged at 10000 x g for 1 minute to remove all traces of ethanol and placed into clean micro centrifuge tube and 200 µL of preheated elution buffer added directly into column membrane and stand at room temperature for 2 minutes before it was centrifuged at 5000 x g for 1 minute to elute DNA and stored at 4 ***°C***.

PCR for *H. pylori* 16S rRNA gene detection

In this study 16S rRNA, was used for the detection of *H. pylori.*

The extracted DNA of the biopsy specimens was amplified with the primers 16S rRNA-f (forward 5GCGCAATCAGCGTCAGGTAATG3) and 16S rRNA-r (reverse: 5GCTAAGAGATCAGCCTATGTCC3). These primers amplify a fragment of 470 bp of 16S rRNA.

PCR was carried out in 20 µL reaction mix which include 1 µL of forward and 1 µL of reverse primer, 14 µL of reaction mixture which contain 7 µL distilled water, 3 µL buffer, 3 µL $MgCl_2$, 1 µL dNTPs and 4 µL of extracted DNA. The amplification reaction was carried out in thermo cycler with cycling program consisting of an initial denaturation step at 94 ***°C*** for 10 minutes, followed by 30 cycle of denaturation at 94 ***°C*** for 1 minute, annealing at 53 ***°C*** for 2 minutes, extension at 72 ***°C*** for 3 minutes and final extension step at 72 ***°C*** for 5 minutes.

Amplified PCR products were electrophoreses in 1.5 % agarose in TBE buffer and stained with Ethidium bromide before being visualizing approximately 470 bp band size compared with 100bp DNA ladder (solisbiodyne, cat No.07-11-00005) under UV light using Gel documentation system (UK, model (Ingeniusl).

Ethical consideration

Approval for this study was obtained from ethical committee of the faculty of medical laboratory sciences, Al-Neelain University and Alribat Teaching hospital, consent was taken from each patients.

Results

A total of 50 patients with gastrodudenitis participated in our study. There were 39 (78%) males and 11(22%) females. The minimum age was 20 years and the maximum was 79 years.

Prevalence of *H. pylori* DNA in patients with gastroduodenitis and peptic ulcer were 52% in our study.

Out of 50 specimens tested 26 were positive (52%) and 24 were negative (48%) by using PCR. Out of 26 positive by PCR 22 were positive by RUT and out 24 negative by PCR 21 were negative by RUT.

Table 1 shows the comparison between results of RUT and PCR. The total number of those who were both positive for RUT and PCR (true positive) was 22(44%), those who were positive for RUT but negative for PCR (false positive) were 3 (6%), those that were both negative for RUT and PCR (true negative) were 21(42%), while those that were positive for PCR but negative for RUT (false negative) were 4 (8%). The sensitivity, specificity of RUT were 84.6% and 87.5% respectively(Fig. 1).

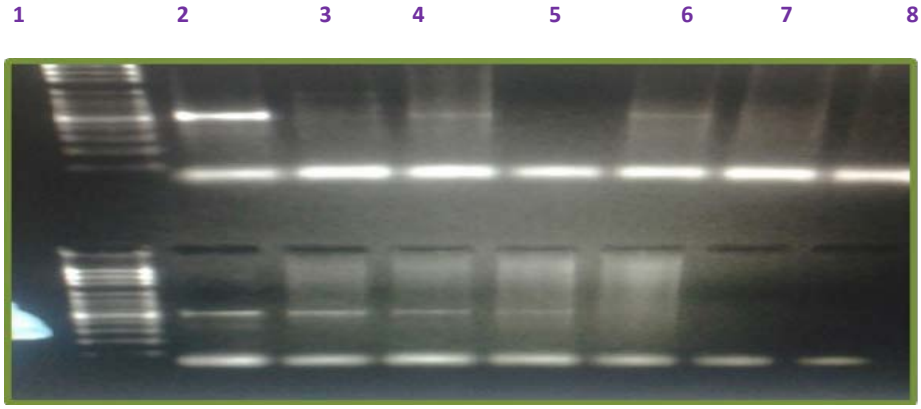

Figure 1 Gel electrophoresis of *H. pylori* DNA PCR product. Lane no. 1 contains 100-bp DNA ladder. Lane no. 2 contains control positive, other lanes 4,6 contains positive samples (band appear at 520 bp) .

Table 1: comparison between RUT and PCR

Test		PCR		Total
		Positive	Negative	
RUT	Positive	22(44%)	3(6%)	25(50%)
	Negative	4 (8%)	21(42%)	25(50%)
Total		26(52%)	24(48%)	50(100%)

Discussion

Our study was aimed to evaluate the diagnostic accuracy of RUT in the diagnosis of *H. pylori* infection, using PCR as gold standard method.

The prevalence obtained in our study 52% which were similar to Hiba Nugdalla Allah Abdorahman *et al* (2013)[6] in Sudan and Ousman *et al* (2011) [7] in West Africa, where the rate of *H. pylori* infection were reported as 52.5% and 52% respectively .while weren't similar to Abbas Bakhit Mohammed Rahama *et al* (2013)[8] in Sudan and Al-Sulami *et al* , (2013)[9] in Iraq, where the rate of *H. pylori* infection were reported as 90% and 66.5% respectively .

The sensitivity and specificity of RUT in our study were (84.6%) and (87.5%), respectively, which weren't similar to Jemilohun *et al*, (2011)[10] in Nigeria were reported the sensitivity and specificity 93.3% and 75.6% respectively and also weren't similar to Brooks *et al*, (2004) [11]in New Zealand were reported the sensitivity and specificity 98% and 100% respectively.

False-positive results can occur owing to reflux of alkaline bile into the stomach [12] .The presence of blood may also adversely affect the performance of RUT leading to false negative results. This is due to the buffering effect of serum albumin on the pH indicator, rather than by a direct inhibition of the urease activity [13].

This variation of results could be attributed to ethnic differences and the small sample size used in our study. Abuse of NSAID in Sudan may cause an increase in the incidence of the gastritis without involvement *H.pylori.*

The relatively lower values obtained in our study compared to those of Jemilohun *et al*, (2011) [10]. and Brooks *et al*, (2004) [11]could be explained by the fact that They used more than one diagnostic method as reference standard, The implication of this is that any infection missed by one test could be easily picked by the other tests, thereby increasing the number of positive results by the reference standard.

Conclusion

RUT show high specificity and sensitivity, simpler, gives more rapid test results and much cheaper it will serve as good alternative to PCR particularly in resources limited area, Practically overcomes the drawbacks since it is not dependent on the experience and accuracy of individual laboratories as in the case of other biopsy based methods.

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