ISSN: 2279 - 0594

Available Online at www.jbpr.in

Journal of Biomedical and Pharmaceutical Research 2 (5) 2013, 48-51

RESEARCH ARTICLE

MOLECULAR CHARACTERIZATION OF THE 16S RRNA GENE OF *HELICOBACTER PYLORI* ISOLATED FROM STOMACH BIOPSY IN GASTRITIS PATIENTS

Hiba Nugdalla Allah[1], Abdorahman [2], *Walid A. H. Eldaif [2]

[1] B.Sc. Medical Microbiology, Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Al Neelain University, Sudan.

[2] B.Sc. M.Sc. Ph.D. Medical Microbiology, Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, Al Neelain University, Sudan.

Received 11 May 2013; Revised 25 May 2013; Accepted 25 June 2013

ABSTRACT

Background: *Helicobacter pylorus is* now accepted as the most important cause of gastritis in human. There is a growing body of literature on the detection of *H. pylori* 16-s gene in grastritis patients. The study was aimed to determine the frequency of *H .pylori* (16-S) gene, in patients with gastritis at Khartoum State, Sudan.

Methods: Molecular testing for *H. pylori* 16-S gene was done on 40 stomach biopsy using PCR technique. Biopsy specimens was collected by gastroenterologist using endoscopy .one biopsy of inflamed stomach was collected in sterile normal saline and kept at -20^{O}C till used. Extraction was done by using ml jena bioscience kit. The amplification reaction was carried out in thermo cycler machine PCR system with program system consisting of an initial denaturation step at 94^{0} c for 10 minute and 30 cycle of denaturation at 94^{0}c for 1 minute

Results: Twenty one samples (52.5%) out of 40 were positive by PCR, while 19 samples (47.5) were negative.

Conclusion: The frequency of *H. pylori* among endoscopic patients was 52.5%, so the frequency of infection was high.

INTRODUCTION:

Helicobacter pylori, is the main cause of chronic active gastritis, and has major role in development of duodenal ulcer, also associated with but not necessary the cause of gastric carcinoma ASM [(1)]

There are found 80% of chronic gastritis caused by *H. pylori* [(2)]Since the discovery of *Helicobacter pylori* in the 1989 by Warren and Marshal, much has been learned about this gram-negative spiral bacteria and its associated disease states. In 1994, the National Institute of Health (NIH) Consensus Conference recognized *H. pylori* as a cause of gastric and duodenal carcinoma [(3)] *H. pylori* consists of a large diversity of strains, and the genomes of three have been completely sequenced. The genome of the strain "26695" consists of about 1.7 million base pairs, with some 1,550 genes. The two sequenced strains show large genetic differences, with up to 6% of the nucleotides differing [(4)] . Study of the *H. pylori* genome is centered on attempts to understand pathogenesis, the ability of this organism to cause disease. Approximately 29% of the loci are in the "pathogenesis" category of the genome database. Both sequenced strains have an approximately 40 kb-long Cag pathogenicity island (a common gene sequence believed responsible for pathogenesis) that contains over 40 genes. This pathogenicity island is usually absent from *H. pylori* strains isolated from humans who are carriers of *H. pylori* but remain asymptomatic[(5)] .The cagA gene codes for one of the major *H. pylori* virulence proteins. Bacterial strains that have the cagA gene are associated with an ability to cause ulcers [(6).] The cagA gene codes for a relatively long (1186 amino acid) proten. The cag pathogenicity island (PAI) has about 30 genes, part of which code for a complex type IV secretion system. The low GC content of the cag PAI relative to the rest of the helicobacter genome suggests that the island was acquired by horizontal transfer from another bacterial species[(4)] .16S rDNA sequencing has played a pivotal role in the accurate identification of bacterial isolates and the discovery of novel bacteria in clinical microbiology laboratories. For bacterial identification, 16S rDNA sequencing is particularly important in the case of bacteria with unusual phenotypic profiles, rare bacteria, slow-growing bacteria, uncultivable bacteria and culture-negative infections.[(7)] .Researchers are not knows how people contract *H. pylori*, but they think it may be through food water[(2)] . Researchers have found *H. pylori* in the saliva of some infected people. So the bacteria may also spread through mouth-to –mouth contacted such as kissing [(3)] *H. pylori* can be transmitted from person to person through close contact and exposure to vomit.

*Corresponding author: Walid A. H. Eldaif | Email: eldaifwah@hotmail.com

MATERIAL AND METHOD:

This is descriptive cross sectional study done on patients conduct in April to May 2012.Biopsy specimens was collected by gastroenterologist using endoscopy .one biopsy of inflamed stomach was collected in sterile normal saline and kept at -20 ^{O}C till used. Extraction was done by usingml jena bioscience kit, according to manufacture instruction 5-10 mg of frozen tissue was transferred to 1.5 micro tube, containing 0.3 ml of cell lysis solution , then 1.5 ml protienase K solution was added to lysate and mixed by inverting several times, then incubated at 55^{O}C overnight, then 100 ml of protein perception solution was added to cell lysate then mixed by vortexing for 20 second and centrifuged atb1500bg forb3 minute, the supernatant was transferred to clean 1.5 ml microtube containing 300 ml of more than 99% isopropanol, and sample was mixed by inverting gently 50 times, followed by centrifugation at 15000g for minute , the DNA was visible as pellet that range in color from off-white to light green. The supernatant was discharged and the tube was drained on clean absorbent paper. Then 300 ml of ethanol was added and the tube was inverted several times to wash the DNA pellet. Centrifuged at 15000 g for 1 minute. The ethanol was discharged carefully then air dried at room temperature for 10-15 minute, finally DNA was eluted by adding 50-100 ml of DNA hydration solution, kept at -20^{O}C till used. Primer designed 16 S rRNA forwared (5GGTAAGAGATCAGCCTATGTCC3) and 16srRNA reverse (5GCTAAGAGATCAGCCTATGTCC3) was used. The primer produced 470 bp band sizes in case of *H. pylori* infection. Prepration of 20 ml PCR master mix this was done by adding 7.5 ml D.W ML buffer, 3ml $MgCl_2$, 1ml dNTPs, 1 ml of forward and 1 ml of reverse primer, 0.5 ml Taq polymerease and 4ml sample DNA. The mixure was vortexed. The amplification reaction was carried out in thermo cycler machine PCR system with program system consisting of an initial denaturation step at 94^{0} c for 10 minute and 30 cycle of denaturation at 94^{0}c for 1 minute , annealing at 53^{o}c for 2 minute, extention at 72^{o}c for 3 minute and final extention step at 72^{o}c for 5 minute. Absence or presence of PCR products was visualized by electrophoresis. The PCR products were loaded in 1.5% Agarose gel. The gel was prepared as followed: 0.53g of the agarose was added to 35 ml of 5x Tris Borate EDAT buffer (7g boric acid, 13.75g Tris base, and 0.465g EDTA (in a 125 ml of distilled H_2O , pH adjusted to 7.5). the mixure was heated until a homogenous solution was formed, then 7 µl of ethedium bromide was added to the mixure.35 ml of the gel were added to the gel box and 5 µl of genomic DNA samples loaded into the agarose gel wells after mixing with loading dye. After that The gel was run at 50 V for 30 min. The gel was then examined in Gel documentation system INGeNius.

ETHICAL CONSIDERATION:

Approval was taken from Al-Neelain Ethical committee to done this study and from the s Hospital's Ethics Committee, also informed consent was obtained from all patients.

STATISTICAL ANALYSIS:

Collected data were analyzed using the Statistical Package for Social Science (SPSS) for the analysis of *H. pylor*i in gastritis patients.

RESULTS:

Forty biopsy specimens were included in this study for the presence of H. *pylori 16.S* gene using PCR. 21 samples (52.5%) out of 40 were positive, whereas 19 samples (47.5%) were negative.

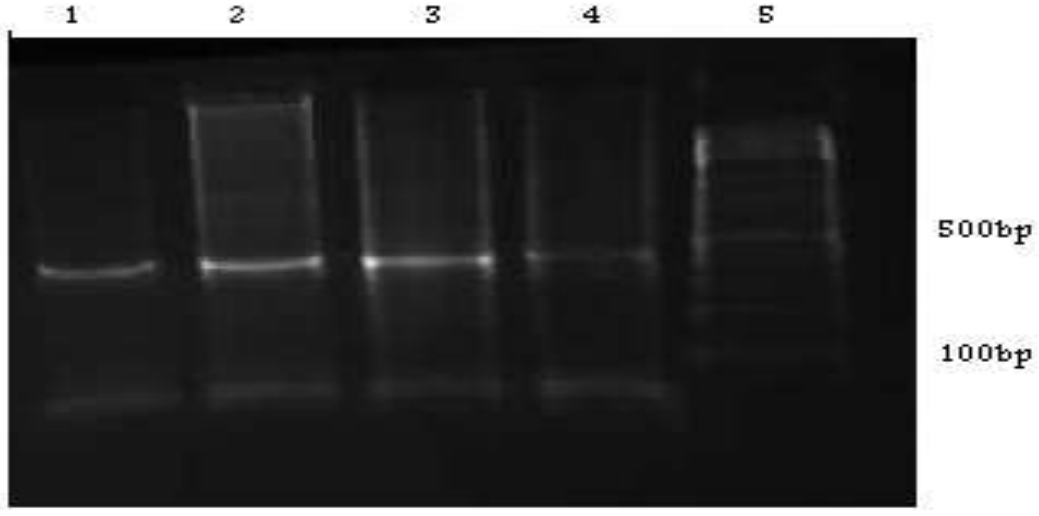

Figure (3.1): PCR results of *H. pylori* showed Lanes (1, 2, 3, and 4) positive samples with 470bp in size,lane (5) is MWt marker.

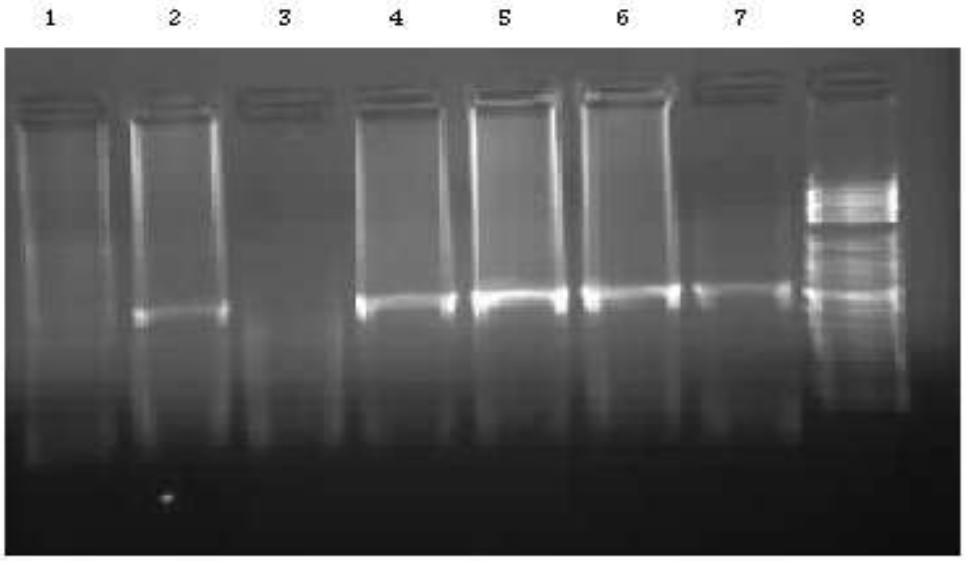

Figure (3.2) PCR results of *H. pylori* showed: Lane (1&3) negative samples, and Lane (2, 4, 5, 6, and 7) positive samples with band size 470bp. Lane (8) is MWt marker.

Table (1.1.): The relation between PCR result & *H. pylori* 16.S gene among the study group.

PCR result	Number	Percentage
Positive	**21**	**52.5%**
Negative	**19**	**47.5%**
Total	**40**	**100%**

DISCUSSION:

Helicobacter pylori, is the main cause of chronic active gastritis, and has major role in development of duodenal ulcer, also associated with but not necessary the cause of gastric carcinoma ASM. There are found 80% of chronic gastritis caused by *H. pylori* in addition to another form of chronic gastritis are seen, which include eosinophilic gastric associated with food allergy and reflux gastric [2]. *H. pylori* usually affect the antrum and pylori canal, and causing superficial inflammation involving the upper half of the mucosa [3].

Our study showed that 21(52.5%) out of 40 were positive by PCR, similar finding was found in study done in Nigeria by Jemilohun et al, (2011) [8] they detected a frequency 45.3% from total population 83subjects. Another study done in Newziland by brooks, et al. (2004) who detected 59 positive sample out of 134 subjects [9]

CONCLUSION:

The frequency of *H. pylori* among endoscopic patients was 52.5%, so the frequency of infection was high.

RECOMMENDATION:

Because up 70% of gastric ulcers caused by *H. pylori*, detection and treatment of infected person is essential to prevent consequence complication such as gastric carcinoma. Strategy for the prevention of *H. pylori* infection include education about the danger of *H. pylori* and is adverse complications should be established.

REFERENCES:

1. Collee, J. G., Fraser, A.G., Marmion, B. P., and Simmons, A. (1996), Practical Medical Microbiology, 14th Edition. 4: 441.
2. Holstonk and Calom. J. (1997). Helicobacter. An engineering pathogen *European J.* of clinical investigation.24:501-510.
3. Flier and Underhil (1990). Focus on *H. pylori*. New England J. of medicine.322:909-16.
4. Kusters JG, van Vliet AH, Kuipers EJ (July 2006). " Pathogenesis of *Helicobacter pylori* infection. *Clin Microbiol Rev* 19 (3): 449–90.
5. Baldwin DN, Shepherd B, Kraemer P, *et al.* (2007). "Identification of *Helicobacter pylori* genes that contribute to stomach colonization". *Infect Immun* 75 (2): 1005–16.

6. Broutet N, Marais A, Lamouliatte H, *et al.* (2001). "cagA Status and eradication treatment outcome of anti-*Helicobacter pylori* triple therapies in patients with nonulcer dyspepsia". *J Clin Microbiol* 39 (4): 1319–22.

7. Woo PC, Lau SK, Teng JL, Tse H, Yuen KY.State Key Laboratory of Emerging Infectious Diseases; and Research Centre of Infection and Immunology, The University of Hong Kong, Hong Kong, China.

8. Jemilohun1, A.C., Otegbayo2, and J.A., *et al.* (2011) diagnostic accuracy of rapid urease test in diagnosis of *H. pylori* infection in patients with dyspepsia. *AFR. J. CLN. EXPER. MICROBIOL*. 12(2):62-66.

9. Brooksa, and D. Ahmeda, *et al.* (2004) Diagnosis of *Helicobacter pylori* infection by polymerase chain reaction: is it worth it? , *Diagnostic Microbiology and Infectious Disease* 50 (2004) 1–5.

