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Research Article
Prevalence of *H. pylori* Infection among Endoscopy Patients at a Hospital in Makurdi, Nigeria, Using 16s rRNA PCR

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Abstract

Background and Objective: *Helicobacter pylori* infection is currently recognized worldwide as one of the most common chronic bacterial infections and causes of gastritis, peptic ulcer, gastric cancers and gastric malt lymphoma. It is a debilitating infection, reducing drastically the quality of life. **Materials and Methods:** The *H. pylori* were isolated from gastric biopsy samples obtained from the antrum of 80 consenting patients referred for endoscopy at Benue state University Teaching Hospital, (BSUTH), Makurdi, Benue state, Nigeria, placed in Brain Heart Infusion (BHI) broth and tested by both universal bacterial Polymerase Chain Reaction (ubPCR) and 16S rRNA gene (specific to *H. pylori*) PCR . Genomic Deoxyribonucleic Acid (gDNA) was extracted from the biopsy samples using *ReliaPrep* spin column method and used for singleplex PCR. **Results:** Out of the 80 biopsy specimen collected, 24 (30%) had *H. pylori* infection as detected by 16S rRNA gene PCR. Using Chi-Square ((χ^2) test, there was no significant association between *H. pylori* infection and gender (χ^2 = 2.360, p = 0.124). However, significant associations were found between infection and age (χ^2 = 11.494, p = 0.009) and location of subjects (OR = 2.922, 95% CI: 1.027-8.313, p = 0.040). The findings of this study show high prevalence of *Helicobacter pylori* in gastritis patients in Makurdi, Benue state. **Conclusion:** Medical personnel handling cases of gastritis should consider *H. pylori* infection in the first line of treatment, especially as most illnesses are treated presumptively and symptomatically in most hospitals in this region, without recourse to rigorous laboratory tests. Further, education and public awareness highlighting the relevance of hygiene should be stepped up to help reduce infection rates.

Key words: Endoscopy, gastric biopsies, *Helicobacter pylori*, PCR, 16S rRNA

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Helicobacter pylori is a Gram-negative micro-aerophilic spiral bacterium. It infects more than half of the world's human population with prevalence ranging from 25% in developed countries to more than 90% in developing countries[1]. The principal reason for this variation may involve socio-economic differences between the populations, a lack of proper sanitation, safe drinking water, basic hygiene as well as poor diets and overcrowding[2].

Infection with the bacterium causes chronic gastritis, peptic ulceration, gastric cancers and gastric Mucosa Associated Lymphoid Tissue (MALT) Lymphoma[1]. *Helicobacter pylori* has been rated as a "class one" carcinogen of the gastrointestinal tract by the World Health Organization in the same category as cigarette smoke to lung cancer[3].

Since the discovery of *H. pylori* in the early 1980s, different techniques have been developed for its diagnosis in clinical specimen. These tests may be invasive or non invasive[4]. The polymerase chain reaction (PCR) has increasingly been described as the latest 'gold standard' for detecting some microbes. It is the most commonly used nucleic acid amplification technique for the diagnosis of infectious diseases surpassing the probe and signal amplification methods[5]. It is a highly sensitive method in clinical diagnosis and has been widely used in a number of epidemiological studies[6,7]. Molecular techniques have helped in detecting *H. pylori* from water, gastric biopsies, stool, luminal aspirates, vomitus, saliva, dental plague and other oral cavities that can provide prolonged habitat for the organism as it gains its way to infect the host[8-10].

The PCR proton that amplifies the *ureC* (glm*M*) gene of *H. pylori* has been targeted to detect *H. pylori* due to its highly conserved nature in this organism[11]. However, several other genes have also been targeted in the detection of *H. pylori*, the 23S rRNA gene, 16S rRNA, *ureA* and *ureB* among others[12,13].

While *H. pylori* has a single copy of adhesion or (*ure*A) gene, reports suggest that PCR with 16S rRNA primers is more sensitive in detecting *H. pylori* than PCR with *ure*A primers[12]. While the 16S rRNA gene is also a highly conserved gene and has been targeted in several studies, micro-organisms often contain more than one copy of the 16S rRNA gene and if this is not taken into account during analysis, there will be an over representation of species containing more than one 16S rRNA gene. Furthermore, a 5% heterogeneity in 16S rRNA genes within the same organism has been reported[14].

Endoscopy backed by gastric mucosal biopsy methods, histology, culture or urease tests are forms of invasive tests used, while non-invasive tests include Enzyme Immuno-sorbent Assay (ELISA), Urea Breath Test (UBT), *H. pylori* stool Antigen Test (HPSTAR) and latex agglutination test[8,15].

The burden of *H. pylori* disease is such that the infected individuals may go through life taking drugs, avoiding certain foods and drinks because of the high un-likely hood of a cure[16]. Although extensive research has been carried out on *H. pylori*, the research so far in Nigeria has tended to focus on its prevalence in certain locations. Moreover, little literature exists on the diagnostic methods used in detecting the organism in Makurdi, Nigeria.

This study will help in proper diagnosis and possible treatment which will bring a big relief from the burden the infection places on patients.

MATERIALS AND METHODS

Ethical approval: Ethical approval was obtained from the Health Research Ethics Committee of the Benue state University Teaching Hospital, Makurdi. Participants in the study were informed of details of the study and their consent for enrolment and participation sought.

Sample size determination: Sample size was calculated using Raosoft (2014) Sample Size Calculator. At 0.05 alpha level of significance, 95% confidence level, a patient population size of 99 and previous prevalence of 50%, a sample size of 80 was obtained.

Patient recruitment: Subjects were patients who had various *H. pylori*-associated dyspeptic symptoms including epigastric pain, fullness, vomiting, nausea and flatulence. Patients who had dyspepsia and required endoscopy as part of diagnosis for dyspepsia were enrolled in the study. Patients without symptoms of dyspepsia but had been on antibiotics in the last 3 months were excluded from the study.

Sample collection: A gastroenterologist performed endoscopy on informed-consenting participants. Gastric biopsy samples were taken from the antrum. Pieces of tissue samples were collected into sterile McCartney bottles containing Brain Heart infusion broth with 1.5% glycerol and stored in the freezer at -20°C within 2 h of collection until transported to the Laboratory in ice packs for analysis.

Genomic DNA (gDNA) extraction: The genomic DNA was extracted using ReliaPrep gDNA miniprep kit obtained from

Promega, Southampton, United Kingdom. Genomic DNA extraction was carried out according to standard protocol, using 200 µL of macerated tissue samples. Extracted DNA was checked for purity at 260/280 nm using a Bio-photometer (Eppendorf, Germany). Nuclease free water was used as blank. Extracted genomic DNA was labelled and used for further tests immediately or stored at 4°C.

Singleplex PCR for *H. pylori* detection

Universal primers for detection of bacteria: This PCR is designed to detect any bacteria from any sample matrix. The primer pair is based on 16S rRNA gene and it amplifies 181 bp product size. Water was used as no template control and *E. coli* DNA sample as positive control. Primer sequences used were as follows[17]:

- **337F-16S rRNA:** GAC TCC TAC GGG AGG CTG CAG
- **518R-16S rRNA:** GTA TTA CCG CGG CTG CTG G

The PCR machine (Eppendorf) was programmed to run as follows: 95°C for 3 min, 95°C (15 sec), 58°C (30 sec), 72°C (30 sec), 72°C (3 min, 35 cycles). The gel was removed from the tank containing the buffer after 30 min and viewed under ultraviolet (UV) light. Positive bands had 181 bp size products.

Specific primers for detection of *H. pylori*: The forward primers 16S rRNA-F and the reverse primers 16S rRNA-R were spun to bring the powder to the bottom of the vial. The primer (Eurofins mwg/operon Germany) sequences were[17]:

- **16S rRNA-F:** GGAGGATGAAGGTTTTAGGATTG (23)
- **16S rRNA -R:** TCGTTTAGGGCGTGGACT (18)

Water was used as no template control and *E. coli* DNA sample as positive control. DNA images were captured using GenoMini Electrophoresis Gel system (VWR, UK).

Statistical analysis: Data obtained from the study were analysed using Statistical Package for Social Sciences (SPSS) version 20, IBM Inc. Chi-square analysis was carried out to measure association between variables.

RESULTS

Table 1 shows that out of the 80 biopsy specimens collected, 24 (30 %) had *H. pylori* as detected by PCR of 16S rRNA gene. There was no significant association with gender as detected by Universal bacteria PCR (Chi-square = 1.966, p = 0.161) and PCR of the 16S rRNA (χ^2 = 2.360, p = 0.124).

Table 1: Distribution of *Helicobacter pylori* by PCR of 16S rRNA Gene

Tests	Frequency (%)
PCR using UBP	
Positive	39 (48.8)
Negative	41 (51.2)
16S rRNA gene PCR	
Positive	24 (30.0)
Negative	56 (70.0)

n: 80, PCR: Polymerase chain reaction, UBP: Universal bacterial primers

Table 2 shows that using universal bacterial PCR, females had a higher infection rate 26(55.3%), while males had an infection of 13(39.4%). This infection rate was however not statistically significant between the sexes (χ^2 = 1.968, p = 0.161). However, though distribution of *H. pylori* by gender, when diagnosis was by PCR of the 16S rRNA gene, again showed more males 13 (39.4%) than females 11 (23.4%) positive for *H. pylori* infection, there was no significant association of infection with sex (χ^2 = 2.360, p = 0.124).

Age wise, individuals <30 years had the highest 9 (69.2%) infection rates, while >55 years had the least 1 (16.7%). There was significant association (χ^2 = 11.491, p = 0.009) between *H. pylori* infection and age according to PCR of the 16S rRNA gene technique.

Table 3 shows that outcome of infection was not dependent on level of education (p = 0.131). With regards to location of subjects, the outcome depended on location indicating that rural people were at more risk of infection compared to urban people (OR = 2.922, 95% CI: 1.027 -8.313, p = 0.040).

DISCUSSION

The higher rates of infection found by diagnosis with Universal bacterial PCR (ubPCR) are not a surprise as the PCR of the 16S rRNA gene is a more sensitive and specific tool for *H. pylori* infection.

The test of association between gender and infection rate showed that *H. pylori* was not significantly related to patients' gender. This implied that *H. pylori* infection is not restricted to any gender but sexes are equally susceptible to infection. Though several other studies have reported higher prevalence of *H. pylori* infection in both males and females than found in this study, no significant association with either sex is reported[18,19]. In Nigeria, a prevalence of 80.4% in Kaduna, Kaduna state has been reported [20]. This discrepancy

Table 2: Distribution of *Helicobacter pylori* by sex and age using PCR of 16SrRNA Gene

Test/sex/age	Positive (%)	Negative (%)	Total (%)	χ^2-value	p-value
UB PCR					
Female	26 (55.3)	21 (44.7)	47 (100)		
Male	13 (39.4)	20 (60.6)	33 (100)	1.968	0.161
Total	39 (48.8)	41 (51.2)	80 (100)		
16S rRNA PCR					
Female	11 (23.4)	36 (76.6)	47 (100)		
Male	13 (39.4)	20 (60.6)	33 (100)	2.360	0.124
Total	24 (30.6)	56 (70.0)	80 (100)		
<30	9 (69.2)	4 (30.8)	13 (100)		
31-43	6 (22.2)	21 (77.8)	27 (100)		
44-56	8 (23.5)	26 (76.5)	35 (100)		
>57	1 (16.7)	5 (83.3)	6 (100)	11.491	0.009
Total	24 (30.0)	56 (70.0)	80 (100)		

UB PCR: Universal bacterial polymerase chain reaction

Table 3: Distribution of *Helicobacter pylori* infection in relation to educational qualification and location of subjects

Variables	Number (%)	Number positive (%)	Number negative (%)	χ^2-value	p-value
Education					
No education	6 (7.5)	1 (16.67)	5 (83.33)		
Primary education	8 (10.0)	5 (62.50)	3 (37.50)		
Secondary education	16 (20.0)	7 (43.75)	9 (56.25)	4.060	0.131
Post secondary education	50 (62.5)	11 (22.00)	39 (78.00)		
Total	80 (100.0)	24	56		
Location					
Rural	21 (26.25)	10 (47.62)	11 (52.38)		
Urban	59 (73.75)	14 (23.73)	45 (76.27)	4.210	0.040
Total	80 (100.00)	24	56		

could be as a result of the differences in sample sizes of these studies compared with the enrolees in this study, especially as only patients for endoscopy qualified for inclusion in this study.

The finding that age group <30 years had the highest rate of infection, while 57 years and above had the least agrees with observations that *H. pylori* infection occur among middle aged subjects and the highly productive age groups in societies[21]. Social activities among could be responsible for the observed high rate of infections in these age groups. This result however, contradicts a report that gastric ulcers tend to affect the older age groups, with a peak incidence between 55 and 70 years[22].

The infection was independent of level of education. This could mean that high levels of education, which should result in greater knowledge acquisition would probably have little impact on *H. pylori* infection if not correctly applied. Though, according to Ahmed *et al.*[23], this result probably reflects infection acquired in childhood and borne throughout life. This negates the finding of the peak levels of infection in certain age groups made in this study as this would mean that all age groups should have equal chances of infection with no particular group having higher prevalence and greater burden of infection.

The significant association between the infection and location of subjects is probably a reflection of social status, standards of living, levels of hygiene and available amenities, such as safe pipe-borne water, etc as patients from rural areas appeared to be at higher risk of infection than their urban counterparts. A similar finding was made in Brazil, where persons from a rural area were more infected than those in an urban area[24].

CONCLUSION

This study found high prevalence of *Helicobacter pylori* in gastritis patients in Makurdi, Benue state. The infection was not associated with gender or educational qualification. There was however significant association with age and location.

In further studies, multiple biopsies should be taken from the antrum and corpus of patients to take care of patchy distribution of the organism and reduce uncertainty in diagnostic results.

Education of the masses highlighting the relevance of good hygiene and sanitation in form of seminars, pamphlets, posters, radio and television messages or group discussions and town criers, especially in villages, need to be encouraged and stepped up to help reduce infection rates.

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