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Detection of *Helicobacter pylori* from Biopsies Obtained from Patients Referred for Endoscopy at Benue State University Teaching Hospital Makurdi, Benue State Nigeria Using Enzyme-linked Immunosorbent Assay (ELISA) Method

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Abstract
Helicobacter pylorus (H. pylori) is a Gram-negative bacterium and the cause of gastrointestinal diseases such as chronic gastritis, gastric ulcers, lymphoma and adenocarcinoma and it remains a public health challenge. This study aimed at detecting *H. pylori* among patients referred for endoscopy at the Benue State University Teaching Hospital, Makurdi, Benue State, Nigeria. The research methodology involved the analysis of biopsies obtained from patients undergoing endoscopy in the hospital. The detection of *H. pylori* antigens was achieved using the Enzyme-linked Immunosorbent Assay (ELISA) method. Out of the eighty (80) biopsy samples collected, 4 (5.0 %) were positive for *H. pylori* while 76 (95.0) were negative. When the occurrence of gastritis was linked with the ELISA technique used in detecting *H. pylori* infection, there was no association between gastritis and detection of *H. pylori.* (χ^2=7.018; p = 0.008). Out of the eighty patients examined, 33 (41.25 %) were males and 47 (58.75 %) were females. *Helicobacter pylori* infection was not significantly associated with sex. (χ^2= 0.133; p= 0.715). This study contributes to the distribution of *H. pylori* in the investigated population as revealed by ELISA method that detected *H. pylori* antibodies specificity for *H. pylori*. Regarding the sensitivity and specificity, ease of work as well as its economic status, ELISA technique can be employed in the diagnosis of *H. pylori* infections. Data obtained from this study would help to inform future research endeavors and may serve as a basis for the development of targeted interventions aimed at mitigating the impact of *H. pylori* infections in the study area and beyond.
Keywords: *Helicobacter pylori* Enzyme-linked Immunosorbent Assay, diagnosis, endoscopy, biopsies.

Introduction

Helicobacter pylorus (H. pylori) is a Gram-negative microaerophilic spiral bacterium [1]. The bacterium known for its association with gastrointestinal disorders such as gastritis and peptic ulcers to more severe conditions like gastric cancer [1,2]. Despite the notable advancements in medical science, the prevalence of *H. pylori* infections continues to raise concerns, especially in patient populations undergoing endoscopy. A lack of proper sanitation, safe drinking water, and basic hygiene as well as poor diets and overcrowding all of which can determine the overall prevalence of infection (Hunt *et al.*, 2010). Prevalence of 82% has been reported in children 5-9 years, 95% in adults of middle age and 70 – 90% in older adults in Nigeria [3]. There is an estimate of more than 80% of Africans infected with *H. pylori* [4]. A study on seroprevalence of *H. pylori* infected patients with peptic ulcer in Kaduna State of Nigeria revealed that out of the 225 patients tested, 181 (80.4%) were positive for *H. pylori* while 44 (19.6%) were negative [5]. A similar study was carried out in Enugu State, Nigeria where out of 103 patients, 63 (62%) were positive [6]. Ranked as a class one carcinogen, *H. pylori* still continues to present itself as a serious health concern [7]. However, no literature has been found on its prevalence in Benue State. *Helicobacter pylorus is* very common in Nigeria as in other developing countries [8]. Disease outcome may be the result of a number of factors including the host, environment and differences in the prevalence or expression of bacterial elements [9]. Since the discovery of *H. pylori* as an important etiological agent in gastritis, and peptic ulcer disease, investigation for this bacterium during endoscopy has become a standard clinical practice to establish active *H. pylori* infection [10].

Globally, *H. pylori* infection remains a public health issue, affecting many individuals and necessitating an urgent exploration of diagnostic methods to inform clinical management [11]. This study addresses the need for precise detection of *H. pylori* in patients referred for endoscopy at the Benue State University Teaching Hospital in Makurdi. The recognition of the fact that accurate and timely identification of *H. pylori* is important for effective clinical decision-making and the implementation of targeted interventions and the potential risks of untreated *H. pylori* infections, ranging from chronic inflammation to the heightened risk of gastrointestinal malignancies [12], a fast and easy method of detecting *H. pylori* among individuals undergoing endoscopy is required. Existing literature underscores the significance of *H. pylori* as a causative organism in various gastrointestinal diseases, necessitating diagnostic approaches to inform clinical practices and public health strategies ([13]. While endoscopy serves as a pivotal diagnostic tool, its efficacy can be significantly augmented through the incorporation of sophisticated diagnostic methods, such as the Enzyme-linked Immunosorbent Assay (ELISA),

thereby contributing to the broader discourse on gastrointestinal health. Hence, the main objective of the current study was to detect *H. pylori* from biopsy specimens from patients referred at Benue State University Teaching Hospital, located in Makurdi, the state capital, using ELISA techniques.

Materials and Methods

Study Area

The research was carried out at the Benue State University Teaching Hospital Makurdi. Makurdi is the Benue State Capital, situated at the bank of River Benue. The town falls between latitude 07 ^{0} 45 ^{1} N and longitude 08 ^{0} 32 ^{1} E with mean elevation of 92 meters above sea level. Benue State is located in the north-central geopolitical region in Nigeria and shares boundary with five other states, namely, Nasarawa to the North, Taraba to the East, Cross River to the South, Enugu and Ebonyi to the South-West and Kogi to the West. The state also shares a common boundary with the Republic of Cameroon in the South- East [14].

Ethical Approval

Ethical approval was sought for and obtained from the Health Research Ethics Committee of the Benue State University Teaching Hospital, Makurdi. All participants had medical referrals for gastric biopsy at the Department of Gastroenterology of the Benue State University Teaching Hospital, Makurdi. Volunteer participants were informed of the details of the study and they all consented to accordingly.

Sample Size Determination

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

Patient Recruitment

Patients were recruited from the Gastroenterology unit of the Benue State University Teaching Hospital, Makurdi, which were herein referred to as subjects. Subjects were patients that had various *H pylori*-associated dyspeptic symptoms including epigastric pain, fullness, vomiting, nausea and flatulence. A Consultant Gastroenterologist at the health facility performed the endoscopy on informed- consenting participants.

Inclusion Criteria

a. Patients had symptoms of dyspepsia
b. Patients required endoscopy as part of diagnosis for dyspepsia
c. Patients informed consent was obtained

Exclusion Criteria

a. Patients without symptoms of dyspepsia
b. Patients who have been on antibiotics for the past three months.

Sample Collection

Gastric biopsy samples were taken from the antrum of the patients. Tiny pieces of tissue samples were collected into two different bottles, sterile McCartney bottles containing Brain Heart infusion broth with 1.5% glycerol and stored in the freezer at -20^0 C within 2 hours of collection until transported to Safety Molecular Pathology Laboratory, Enugu in ice packs to maintain the cold chain, for analysis [15].

H. pylori Antigen Enzyme Linked Immunosorbent Assay (ELISA)

The indirect ELISA technique using the Diagnostic Automation ELISA *H. pylori* antigen kit (Lot No. 1LD5-213) was used to detect *H. pylori* antigens in the gastric biopsy specimens. The biopsy specimen in brain heart infusion broth containing 1.5 % glycerol was homogenized by vortexing. Microwells were coated with purified *H. pylori* antibody specific to it by pipetting 50 µl of the antibody into the wells. Then, 100 µl of sample was dispensed into the wells and 50 µl of *H. pylori* antigen was used as control. The Antigen Antibody mixture was swirled gently to mix and expel bubbles, and incubated at room temperature (18 – 25 °C) for 30 minutes. The excess liquid was discarded and the wells were washed three times with washing buffer (phosphate buffered saline and tween 20) using a multichannel pipette. One hundred microliters (100 µl) enzyme conjugate containing specific antibody for *H. pylori* and horse radish enzyme was added and incubated at 30 °C at room temperature. The enzyme conjugate was then removed from wells and washed three times in washing buffer. Tetra methyl benzathene (TMB) chromogenic substrate (100 µl) was added and incubated at room temperature for 30 minutes. One hundred microliters (100 µl) of stop solution (Hydrochloric acid) was added to stop the reaction. Intense yellow indicated a positive result.
The optical density (O.D) was read at 450 nm with a microwell reader [5].
A standard curve was constructed by plotting optical density (450nm) on the y axis against the concentration of calibrator ng/ml on the x – axis. The software used was the Graphpad prism version 6.02, software for cluster analysis [5].

Statistical Analysis

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

Results and Discussion

Of the eighty (80) biopsy specimens collected, 4 (5.0 %) were positive for *H. pylori* while 76 (95.0) were negative (Table 1).
Table 2 indicates that when the occurrence of gastritis was linked with the ELISA technique used in detecting *H. pylori* infection, there was no association between gastritis and detection of *H. pylori* (χ^2 =7.018; p = 0.008).

Table 3 shows that out of the eighty patients examined in which 33 (41.25 %) were males and 47 (58.75 %) were females, *Helicobacter pylori* infection was not significantly associated with sex (χ^2= 0.133; p= 0.715).

Table 1: Distribution of Helicobacter pylori Infection by ELISA Method (n=80)

Test	Frequency (%)
ELISA	
Positive	4(5.0)
Negative	76(95.0)

Table 2: Yield of *Helicobacter pylori* from Gastritis Patients by ELISA Test

Test	Gastritis (%)	Normal Mucosa (%)	Total (%)	Chi-Square Value	P-Value
ELISA					
Positive	4(100)	0(0)	4(100)	7.018	0.008
Negative	26(34.2)	50(65.8)	76(100)		

Table 3: Distribution of *Helicobacter pylori* Infection by Sex Using ELISA Method

Test/Sex	Positive (%)	Negative (%)	Total (%)	Chi-Square Value (%)	P-Value (%)
ELISA					
Female	2(4.3)	45(95.7)	47(100)		
Male	2(6.1)	31(93.9)	33(100)	0.133	0.715
Total	4(5.0)	76(95.0)	80(100)		

Discussion

The finding agrees with the findings of [15] in Nippon Medical School Hospital, Shanghai, China. The prevalence of H. pylori using ELISA is not in concordance with the findings of [16] who reported that ELISA was excellent with high sensitivity and specificity in detecting *H. pylori* positive cases in Jamaican adults. The test of association between gender and infection rate showed that *H. pylori* was not significantly related to a patient's gender. An implication of this result is that *H. pylori* infection is not restricted to any gender since both sexes were equally susceptible to the infection. Nevertheless, higher prevalence of *H. pylori* infection in either males or females without any significant association with sex have been reported in earlier studies [17,6] even in Nigeria by [5]. The prevalence rate observed in our study also agrees with global trends, underscoring the persistent public health challenge posed by *H. pylori* infections [11]. The prevalence does not agree with the findings of Ozlem *et al* [18] and Tahereh *et al* [19] who recorded higher rates in Turkey and Iran respectively. This could be as a result of differences in geographical location of the patients. The robustness of ELISA as a diagnostic tool was evident in its ability to provide sensitive and specific identification of *H. pylori* antigens. The implications of our findings are not only within the realm of diagnostic accuracy but also in the broader context of informing targeted interventions and public health strategies [13].

Conclusion

The study was undertaken to detect *H. pylori* among patients referred for endoscopy in the Benue State University Teaching Hospital, Makurdi. The use of ELISA method showed a low prevalence of *H. pylori* and its distribution according to gender was not significant. The outcomes presented herein serve as a foundation for future investigations into the dynamics of *H. pylori* infections, with potential implications for refining diagnostic approaches and tailoring therapeutic interventions.

Competing Interests

Authors have declared that no competing interests exist.

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