Prevalence of *Helicobacter pylori* among Suspected Stomach Ulcer Patients AttendingTwo Mission Hospitals in Umuahia, Abia State, Nigeria

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Abstract: *Helicobacter pylori,* a leading cause of gastritis, peptic ulcers, and other gastritis-related diseases in both developed and developing countries, is a significant public health concern in developed and developing countries, with a disproportionately high burden in economically developing countries due to poor sanitation. This study aims to isolate and determine the seroprevalence of *H. pylori* among patients attending two Mission hospitals in Umuahia, Abia State. Samples of blood and stool were collected from ninety patients. The blood was collected aseptically from the antecubital vein using a sterile disposable syringe and needles and allowed to clot for antibody detection using *H. pylori* strip kit (*Helicobacter pylori* Ab Rapid test (Cassette Germany). The isolation of *H. pylori* from the stool samples was done by inoculating onto blood and chocolate agar plates and then incubated in a microaerobic atmosphere at 37^0Cfor 5 days. Identification was based on typical colony morphology, characteristics appearance on gram staining and positive urease, oxidase and catalase tests. A high prevalence of 23.33% was observed in this study. Gender was not significantly associated with *H. pylori* infection (X^2=0.1517, p=0.3712). The highest incidence was observed in the age group 16-26(38%) while the least was recorded with those of 49years and above 3(14.4%). There was no significant association of age with the prevalence of *H. pylori* infection (p=0.661). More cases of *H. pylori* infection was observed with patients from the rural area (16.67%). Out of the 90 participants suspected of having peptic ulcer, 23.33% of them had ulcer that was caused by *H. pylori* Out of the 21 ulcer patients cause by *H. pylori* 16 (76.2%) were both seropositive and culture positive to *H. pylori* while 5 (23.4%) were only seropositive. The prevalence of *H. pylori* infection was low among patients with peptic ulcer attending the two mission hospitals. In order to lessen the disease's impact, health education on transmission channels and risk factors for *H. pylori* infection is emphasized in this line.

Keyword*: Helicobacter pylori* infection, peptic ulcer, prevalence.

INTRODUCTION

For more than a century, it has been known that bacteria are present in the human stomach and other parts of the gastrointestinal tract. However these bacteria were thought to be contaminants from digested food rather than true gastric colonizer (Parsonnet *et al,* 1999). *Helicobacter pylori* (*H.pylori*) organisms have been found in the stomach of human in all part of the world and is commonly isolated from non-human primates as well (Colmand Martin, 2000).

*H. pylori*is a spiral-shaped Gram negative bacterium that is found in the gastric mucous layer or adherent to the epithelial lining of the stomach (Stevenson *et al.,* 2000). It only grows in Microaerophilic or in carbon dioxide-enriched (5-10%) atmosphere and exhibits optimal growth at 35-37^0C (Thirunavukkarasu *et al.,* 2017). The bacterium grows very slowly, requiring 3-4days of growth before colonies become apparent on initial isolation (Sean *et al.*, 2004).

H. pylori is an economically important infection with a worldwide prevalence of 50% (Go, 2002). This infection is mostly acquired during childhood and adult through the fecal-oral and oral route (Malaty *et al.*, 2002, Quaglea and Dambrosio, 2018). Initial infection with this organism is usually silent but symptoms and pathologic changes occur later in life (Michael and Heidi, 2008; Brown, 2000). The clinical conditions and pathological changes associated with *H. pylori* infection include gastritis, gastric and duodenal ulcers, gastric cancers, iron deficiency anemia and idiopathic thrombocytoperic puerperal (ITP) (Franchini *et al.,* 2007). *H. pylori*are diverse bacterial species, and every infected individualharbors aunique strain apart from the strain concordance found in some families (Colmand Martin, 2000).

In the developed countries, 30% to 40% of the population harbor *H. pylori,* while in the developing countries, the prevalence is higher, ranging from 70%-90% (Saad and Chey, 2008). Studies by Bello *et al.*(2018) in Kano, Nigeria,reported an *H. Pylori* prevalence of 81.7%, Aboderin *et al.*(2007) said 73% in Ile-Ife South-west while in Kaduna, Nwodo *et al.*(2009) obtained a prevalence rate of 80.4%. Also, Obiajuru and Adogu, (2013) in Orlu, Imo State reported an *H. Pylori* prevalence of 58%, while Chukwuma *et al.*(2020) in Akwa, Anambra State observed a prevalence rate of 51.4%. Reports from studies conducted in many African Countries kepta 75.4% prevalence rate in Ghana (Baako and Danko, 1996), 91.7% in Egypt (El Dime *et al.,* 2008) and 97% in the Gambia (Secka *et al.,* 2011). A report from China by Shi *et al.*(2008) observed a 62% prevalence rate.

*H. pylori*infection exhibits avaried geographic distribution on local and global scales (Archampong *et al.,* 2015; Pounder andNg, 1995). These variations are mostly socioeconomically driven; factors such as age, gender, genetic predisposition, ethnicity, educational level and sanitation determine the incidence and prevalence of this global infection (Archampong *et al.*2015; Perez-Perz *et al*., 2004). In Western countries, about 50% of the people over 60 years of age are infected, while 20%of individuals below 40 years are infected. Females have an incidence of re-infection (5-8%) than adult males (Awuku, 2017). Most studies suggest that males and females are infected at approximately the same rate, although male sex was a significant risk factor for infection in at least one study (Valliani *et al*., 2013; Broutet *et al* 2001).

In industrialized countries, *Helicobacter pylori* seroprevalence in children younger than five is 1-10%. In contrast, in developing countries, rates of more than 50% are expected in children of the same age group. In industrialized countries, a decrease in the risk of infection is observed in successive generations (a cohort effect) (Yvonne *et al.*, 2001). The acquisition of infection does not appear to be seasonal (Yvonne *et al.*, 2001).

The role of searching for and eradicating*Helicobacter pylori*both in the general populationsas a means of reducing the incidence of gastric diseases cannot be overemphasized. Based on information and personal observations, stomach ulcer possess a considerable health challenge among individuals. However, there has not been any reported study on the prevalence of *H. Pylori* in Abia State; as a result, the gastric colonization by *H. pylori* remains undefined in Abia State. Therefore, this study aimed to isolate and determine the seroprevalence of *H. pylori* from patients suspected of having stomach ulcers.

MATERIALS AND METHODS

Study Area

The study was conducted in Umuahia, Abia State, located in the South-Eastern part of Nigeria. Abia State is bounded in the North by Ebonyi State, specified in the South at South-West by River State, bounded to the East and South-East by Cross River and Akwa Ibom State. It is bounded in the West by the Imo and North West by Anambra. There is one Federal Hospital (Federal Medical Centre Umuahia), twostate Hospitals (Abia Specialist and General Hospital Amachara), two mission Hospitals and a host of Private Hospitals in Umuahia. The specific studied area is Mercy Specialists Hospital, located at Ahiaeke in Umuahia and Madonna Catholic Hospital, situated in Umuahia. These two hospitals have many medical facilities that treat at a subsidized rate that attracts many patients. This study was carried out when the Medical Practitioners in Federal Medical Centers were on strike. The preferred Hospital then were the two Mission Hospitals. Above all, they were more accessible to us.

Study Population

A total of 90 samples were randomly collected from two mission hospitals, including 57 samples from Madonna Catholic Hospital and 33 samples from Mercy Specialist Hospital.

Samples were collected from patients diagnosed with stomach ulcer by the physician through a faecal occult blood test (FOBT). Participants include males and females within five to ninety (5-90) years.

Ethical consideration

Informed consent of each participant was obtained before specimen collection and testing, andEthical approval was also obtained from the Ethical committees of the Hospital. The consent of the administration of the Hospital was also sought.

Sample collection

The samples collected were blood and stool samples. The blood was collected aseptically from the antecubital vein of each patient using a 10ml sterile disposable syringe and needle.It was aseptically transferred into a sterile test tube and allowed to clot for *H.pylori*antibody detection. The stool sample was collected using a clean container (universal bottle), and both samples were collected from each patient. After collecting the samples, they were placed on an ice packand transported aseptically to the Microbiology laboratory of the Michael Okpara University of Agriculture, Umudike (Cheesbrough, 2006).

Confirmation of Ulcer Patients

Stool samples from already diagnosed ulcer patients were collected subjected to a faecal occult blood test (FOBT). A faecal occult blood test strip (Sure-care USA) was used. The test was done following the manufacturer' instructions.

***H. pylori* serum antibody detection**

Following the manufacturer's instructions, antibody detection was performed using *H. pylori* test strip kit (Helicobacter pylori Ab rapid test (cassette) Germany). The patient's serum was separated and centrifuge (centrifuge SM 90–2A microfield instrument, England) at 2000rpm for 5mins to remove any cellular debris from the serum. A drop of the serum sample was placed on an Ab Rapid test (cassette) to detect the presence of 1gG and lgA antibodies to *Helicobacter pylori.*

Isolation of *H. pylori*

The stool samples were place in a test tube containing phosphate-buffered saline and was mixed using a spatula. The isolation of *H. pylori* from the stool sample was done by inoculating (streak method) the homogenized stool sample onto blood and chocolate agar plates. The plates were incubated under microaerophilic (5% oxygen) and hypercapric (5% CO_2) condition at 37 °C for five days (Cheesbrough, 2006).

Identification of *H. pylori*

A single colony was picked from each primary culture plate and identified as *H. pylori*based on typical colony morphology, characteristic appearance on gram staining and positive urease, oxidase and catalase tests (Patrick *et al.,* 2002).

Data Analysis

All data were entered into statistical software package for social sciences (SPSS software version 10). Descriptive statistics was computed to determine frequency and percentage. Chi-square and student t-test were employed to test associations between various characteristics. Statistical significance was set as P-value of < 0.05.

RESULTS

The prevalence of *H. pylori*infection among the study participant was 23.33%. The prevalence among male was 38.0% and among female was 62.0%. However, gender was significantly associated with *H. pylori* infection (X^2 – 0.1517, P-value = 0.0371) Table 1.Also the highest incidence was observed with age group 16-26 years, 8(38.0%) followed by those of 27-37 years 5(23.3%) while the least was recorded with those of 49years and above 3(14.4%).There was no significant association of age group with the prevalence of *H. pylori* infection (P = 0.6611).

Table 2 depicts the geographical distribution of *H. pylori* among patients examined. The patient from urban area had the least incidence (6.67%) while those from the rural area had the highest incidence (16.67%).

The distribution of patients with *H. pylori* ulcer (HPU) and Non-*H. pylori* ulcer (N-HPU) by age group is as given in Table 3.

As observed from the table 23.33% of patient had ulcer caused by *H. pylori* while 76.67% of patient had ulcer that was not caused by *H. pylori.*

Table 4 present the number of occurrence of patients that were only seropositive and those that were both seropositive and culture positive to *H. pylori* screening. Out of the 21 patients that has ulcer caused by *H. pylori*, 5(23.8%) of them were only seropositive while 16(76.2%) were both seropositive and culture positive.

Table 1: Distribution of Laboratory Confirmed *Helicobacter pylori* infection in relation to gender and age group (n=21)

	Prevalence of *H. pylori infection*		
Age group (yrs)	**Male N(%)**	**Female N(%)**	**Total N(%)**
5-15	1 (4.8)	2 (9.5)	3 (14.3)
16-26	3 (14.3)	5 (23.8)	8 (38.0)
27-37	2 (9.5)	3 (14.3)	5 (23.8)
38-48	1 (4.8)	1 (4.8)	2 (9.5)
49-59	0 (0)	1 (4.8)	1 (4.8)
60-70	0 (0)	1 (4.8)	1 (4.8)
>70	1 (4.8)	0 (0)	1 (4.8)
Total	**8 (38.0)**	**13 (62.0)**	**21 (100)**

X^2 = 0.1517 P-value = 0.0371 (P>0.05: Not significant)

Table 2: Rural-urban distribution of*Helicobacter pylori* among patients examined

Region	Hospital	Number Studied	Number Positive	% Prevalence	Number Negative	% Prevalence
Rural	Madonna	26	11	12.22	15	42.31
	Mercy	19	4	4.44	15	21.05
	Total	45	15	16.67	30	33.33
Urban	Madonna	32	2	2.22	30	6.25
	Mercy	13	4	4.44	9	30.77
	Total	45	6	6.67	39	13.33
	Grand Total	90	21	23.33	69	23.33%

Table 3: Distribution of patients with *Helicobacter pylori* and *non- Helicobacter pylori* ulcer by age

Age range	No. of patients tested	No. of HPU infected patient N (%)	No. of Non-HPU infected patient N (%)
0-19	20	9(45)	11(55)
20-39	20	7(35)	13(65)
40-59	20	3(15)	17(85)
60-79	20	1(5)	19(95)
≥ 80	10	1(10)	9(90)
Total	**90**	**21(23.33)**	**69(76.66)**

Table 4: Occurrence of patients with seropositivity only and Seropositive with culture positive to *Helicobacter pylori screening*

Screening Result	Number	Percentage
Culture positive Only	0	0
Seropositive Only	5	23.8
Sero and Culture Positive	16	76.2

DISCUSSION

H. pylori is more prevalent in the poor socio-economic environment because of its faecal-oral transmission mode (Awaku, *et al,* 2017). This study described the prevalence of *H. pylori* among patients, and the result obtained showed that the prevalence of *H.pylori* was as low as 23.33%. A potential explanation for the findings may include the increasing effective measure to eradicate the infection with antibiotics (Aitila *et al.,* 2019). It may also be related to the slightly improved awareness of sanitation among the inhabitants (Khalifa *et al.,* 2010). The prevalence in this study is low compared with findings from other studies. In a study conducted in Gombe, Yobe state by Mustapha *et al* (2011), *H. pylori* prevalence was found to be 77.1%.Mdububa*et al.* (2001) in Ile –Ife reported a prevalence rate of 73% while Jijjani and Umar, (2008) observed a prevalence of 93.3% among peptic ulcer patients in Kano. In other parts of Africa like Ethiopia, Alebie and Keba, (2016) reported a prevalence rate of 71.0%. However, our results are comparable to previous prevalence obtained in other places where the prevalence of *H. pylori* was 24.3% (Aitila *et al,* 2019), 29.9% (Tsongo *et al,* 2015) and 39.8% (Ayodele *et al.,* 2018).

Moreover, age as a factor in the distribution of *H. pylori* infection was statistically observed in this study to have no significant effect ($p = 0.66$). The age group 16-26 carries the highest burden of the infection followed by those of 27-37 years of age. Most of the participants in these two age groups are mostly students attending schools with poor sanitary facilities and also no access to clean drinking water (Mahalanabis *et al,* 1996). This suggests that the poor hygiene at the school increases the chance of infection with *H. pylori* bacteria. The high prevalence among these age group might also be attributed to the fact that these students eat out at road side restaurants that do not maintain full satisfactory hygiene standard.The *H. pylori* prevalence according to age group as observed in this study is in consonant with what was gotten in other studies, where there were increase in prevalence of *H. pylori* at earlier age, then declined in population over 50 years or 60 years in other countries (Ozaydin*et al,* 2013). Contrary to this, some studies opinioned that *H. pylori* prevalence increased with age (Parente *et al,* 2003). The observation in this study that *H. pylori* infectively is high in the younger population suggests that the infection was acquired during childhood and early adolescence, reaching its peak at adulthood, according to the finding of Jaft, (2011) and Karima *et al,* (2006).

However, this study showed that the female population had (62%) higher prevalence than males (38%). The gender effects on the prevalence of *H.pylori* infection in many people varied.Bello *et al.*(2018) reported a higher of *H. pylori* in Males (82.4%) than in females (81.6%). Also, Omosor *et al* (2017) stated that *H. pylori* prevalence was higher in males (55%) than females (51.4%),and the same was reported by valliani *et al.,* (2013) while others reported no difference. The findings of our study do not correspond with the report of Jemilohun *et al.,*(2010); Jemikajah and Okogun, (2014);Awuku *et al.,* (2017); Ayodele *et al.,* (2018), and Chukwuma *et al.*, (2020). There are conflicting findings on whether males or females have a higher prevalence of *H. pylori* infection, but there is no clear link between the two (Lisi and Kehinde, 2003)and (Smith *et al,* 2001).

More so, this study demonstrated that *H. pylori* infection had a higher incidence amongst participants from rural areas (16.67%) than from the urban area (6.67%). This low incidence among participants from the urban areas compared to the rural area could be because our study participants from the urban area have a higher social class, with better socioeconomic standards to avoid known transmission sources such as contaminated water and foods (Chen, *et al.,* 2014).Participants from the rural might be more of individuals from low socio-economic class and are more likely to be associated with a low level of education, including poor health education. They have more tendencies to live in an environment predisposed to faecal contamination of food and water, hence the higher prevalence. The provision of clear portable water to the rural areas will significantly reduce the burden of this infection among them.Furthermore, the result obtained from this study shows that there are other causes of peptic ulcer other than *H.pylori.* This is evident by 76.66% of the participants having peptic ulcer without *H.pylori* culture positive or seropositive.

Nevertheless, this study showed that most of the participants were sero and culture-positive to *H.pylori* screening (76.20%), whereas 23.80% were only seropositive. Serological tests cannot discriminate between previous and current infections. The sero-prevalence assay's 1gG antibody lastsfor up to 3 years or more in the serum even after eradicating the organism (Jemilohun *et al.,* 2010). These could explain why some were only seropositive without significant growth from the culture, and few were negative.Failure of the organism to grow may be due to its reduced viability after patients' prior treatment with antibiotics or proton pump inhibitors (Kulpers *et al.,*1996).Therefore, there is a need to create awareness for the diseases with significant emphasis on the people's food and public health hygiene considering its faecal-oral route of transmission.

CONCLUSION

The prevalence of *H. pylori* infection among the study participants was high (23.33%). There was no significant difference in the gender and age distribution of *H.pylori* infection among the studied population (p = 0.6611). Participants from rural area had a higher prevalence than those from urban area. Majority of the ulcer cases among the patients were not caused by *H. pylori* as there was 76.66% of non-*H. pylori* infected patients. Most of the study population were both seropositive and culture positive to *H. pylori.*

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