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Prevalence and Possible Risk Factors for Helicobacter Pylori Seropositivity among Peptic Ulcerative Individuals in Nnewi Nigeria

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Background: *Helicobacter pylori* colonizes the human gastric epithelium, causing chronic gastritis, peptic ulcer disease and gastric cancer. Recent studies indicate that the host immune response contributes to the disease during infection with this organism.

Objectives: This work was aimed at determining the level of seropositivity of *Helicobacter pylori* in peptic ulcerative individuals.

Method: A cross sectional study involving 179 peptic ulcerative individuals was conducted. Ethical approval was obtained and informed consent of the participants was sought. Questionnaire was administered and 5mls of blood collected into EDTA container. Subject selection was done using systematic random sampling technique. The *H. pylori* seropositivity was determined using ELISA technique.

Result: The prevalence rate for *H. pylori* was 51.4% and the predominant seropositive age group was 24-35 years (22.9%). Age (p=0.00) was found to be significant risk factors for *H. pylori* seropositivity. Females 50(27.9%) were more seropositive to *H.pylori* than males 42(23.5%) though there was no significant relationship between gender and *H. pylori* seropositivity (p=0.281). Moreover, there was no significant relationship between source of drinking water and *H. pylori* seropositivity (p=0.433). Overall, direct borehole water 16(8.9%) and sachet water users 57(31.8%) predominated in the seropositive population.

Conclusion: *H. pylori* constitute a hidden epidemic and many seropositive individuals with peptic ulceration are ignorant of this organism. The results show that *H.pylori* is high among peptic ulcerative individuals in Nnewi.

Keywords: *Helicobacter pylori, peptic, risk factors, seropositivity, prevalence*

Highlights:

- The results from this study show that the seroprevalence rate of *H.pylori* in the study population is 51.4%.
- There was no association between source of water and *H.pylori* but majority of the participants that are *H.pylori* positive do drink satchet water and borehole water which could be contaminated as a result of improper processing of the satchet water, contamination by water vendors or inadequate drilling of the boreholes.

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Introduction

Helicobacter pylori (*H. pylori*) infection is the most common chronic bacterial infection around the world [1]. It has been shown that 50% of adults in developed countries and 90% of adults in developing countries were positive of serum antibodies against *H. pylori* [2]. The critical period at which *H. pylori* is acquired, is during childhood, especially in the developing countries and areas of overcrowding and socioeconomic deprivation[3]. This bacterium is a small spiral Gram-negative organism. Factors important for colonization include motility, environmental sensing, chemotaxis[4], iron acquisition[5], and acid resistance. The pathogen is the main cause of peptic ulceration, gastric adenocarcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma[6]. It is considered that *H. pylori* infection is the most common cause of morbidity and mortality in upper digestive tract diseases.

Currently, the effects of *H. pylori* infection on the development of extra alimentary ailments such as coronary disease, myocardial infarction, idiopathic thrombocytic purpura, iron deficiency anaemia has been shown[7]. However, only 10-15% of those colonized develop disease while 85-90% remain asymptomatic, and pathogenesis depends upon strain virulence, host genetic susceptibility, and environmental cofactors. Virulence factors include the cytotoxin-associated gene (cag) pathogenicity island (PAI), which induces pro-inflammatory, pro-proliferative epithelial cell signaling; the cytotoxin VacA, which causes epithelial damage; and blood group antigen binding adhesin (BabA). Host genetic polymorphisms that lead to high-level pro-inflammatory cytokine release in response to infection increase cancer risk.

The relation between *H. pylori* infection and lifestyle is uncertain, but its intensification in the individual populations is strongly related to economic conditions[2]. Developing countries are at highest risk, due to people living in poor socioeconomic conditions. The increasing risk factor includes: poor sanitary conditions, overpopulation, consumption of raw foods, like food items purchased from street stalls and unsafe water supply sources[8]. Epidemiological studies demonstrate that the incidence of *H. pylori* infection appears to be higher in children than in adults, possibly due to lower standards of personal hygiene in younger populations[3,9] . Humans are the main reservoir of this infection[10,2]. Infected mother and older siblings are important factors for *H. pylori* transmission to children. The transmission routes are oral-oral (by saliva), which prevails in the developed world, faecal-oral (person-to-person or by contaminated water, or maybe food), mainly in the developing countries or gastro-oral (by vomiting and regurgitation).

Methods available for diagnosis of *H. pylori* include: Invasive Methods such as Endoscopic diagnosis, microscopic examination of histological sections, culture of biopsy specimen, molecular detection of *H.pylori* using polymerase chain reaction (PCR), Rapid Urease Test. Non-Invasive method such as Urea Breath test, Antibody test using either Enzyme Linked Immunosorbent Assay (ELISA) technique or Immunochromatography test (ICT) technique, *H.pylori* Stool antigen test[11].

Methods

A cross sectional study was conducted among 184 peptic ulcerative individuals randomly selected from the medical outpatient clinic and internal medicine clinic of Nnamdi Azikiwe university (NAUTH), Nnewi using the systematic random sampling technique. Ethical approval (with approval number: NAUTH/CS/66/VOL8/31) was obtained from the ethics committee of NAUTH. The participants were diagnosed of peptic ulcer by the physician. Informed consent was obtained from the participants.

Subjects included in the study were aged from fifiteen to seventy years, having persisitent or recurrent abdominal pain or discomfort and with at least two of the symptoms of the epigastric pain and associated symptoms such as bloating, nausea, flatulence and anorexia. All subjects that are pregnant, outside the age of 15-70, currently on antibiotics treatment and do not present any sign of peptic ulcer were excluded. Questionnaires include data on participants demography, symptoms of peptic ulcer, preferred eating habits, source of drinking water and antibiotics use.

Five (5) milliliters of blood was drawn from the participants using 5ml syringe and was dispensed into a plain container. The serum was separated and stored at -20^{0}c for Enzyme Linked Immunosorbent Assay (ELISA) *H. pylori* assay. Assay for H.pylori IgG antibodies in patients sample was according to the manufacturers of the test kits, using the principle and technique of ELISA. The ELISA test was performed using Mindray ELISA machine

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(Shenzhen, China) and the *H. pylori* IgG ELISA kit by Biochem incorporated (Canada).

Purified *H.pylori* antigen is coated on the surface of micro wells. Diluted patients serum is added to the wells and the *H.pylori* IgG-specific antibody, If present, binds to the antigen. All unbound materials are washed away. Enzyme conjugate is added, which binds to the antigen-antibody complex. Excess enzyme conjugate is washed off and substrate and chromogen are added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG-specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls[12]. The data was statistically analyzed using SPSS version 20. Values were expressed as mean±standard mean error. The student's t-test and chi square were used and considered significant if p-value < 0.05.

Results

The seropositivity for H.pylori infection was present in 92 and absent in 87, 5 participants had indeterminate results and were not included in the study population. Figure 1 shows the incidence of seropositivity for *Helicobacter pylori* in peptic ulcerative subjects. Results indicate that a greater percentage (51.4%) of subjects tested positive for *H. pylori*. On the other hand, 48.6% tested negative for *H. pylori*. However, no statistical difference (p = 0.709) was observed between the seropositivity and seronegativity for *H. pylori*.

Table 1 shows the frequency distribution of subjects according to their age groups and *helicobacter pylori* statuses. Data indicate that age group "25-34 years" had the greatest percentage, 44.6% (n = 41) of seropositive subjects, followed by subjects aged 35-44 years, 17.4% (n = 16). Age group ≥65 years had the least percentage of seropositivity. Seronegativity was greatest (29.9%) in age group 35 – 44 years and lowest (2.3%) in age group 25-34 years. Chi-square test (χ^2 = 51.87) shows significant association (p < 0.001) between age and *H. pylori* status of subjects.

Table 2 reveals the frequency distribution of subjects according to their sex and *helicobacter pylori* statuses. Results indicate that a greater percentage of females tested both positive (54.3%) and negative (59.8%) for *H.pylori* than the males (positive, 45.7% and negative, 40.2%). Chi-square test (χ^2 = 0.536) indicated no significant association (p = 0.281) between sex and *H. pylori* status of subjects.

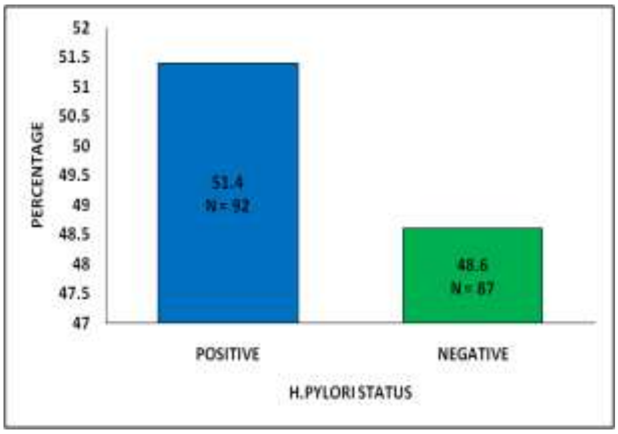

Figure 1. The incidence of seropositivity for *Helicobacter pylori* in peptic ulcer subjects

Table 3 shows the frequency distribution of subjects according to their *helicobacter pylori* statuses and sources of their drinking water. Results indicate that majority, (57 (62%)) of the seropositive subjects were those who drink 'satchet' water, followed by those who use borehole water (16 (17.4)). Subjects who use rain water had the least percentage (1.1%) of seropositivity for *H.pylori*. Subjects who use 'satchet' water also had the highest percentage (49.4%), while those who use rain water also had the least percentage (2.3%) of seronegativity for *H.pylori*. Chi-square test (χ^2 = 4.86) indicated no significant association (p = 0.433) between source of water and *H. pylori* status of subjects.

Table 4 shows the frequency distribution of subjects according to their *helicobacter pylori* statuses and eating habit. Data show that those who eat homemade foods had greater percentage (62%) of seropositivity compared to those who eat outdoor (38%). The same trend was also observed in incidence of seronegativity status of subjects (homemade, 58.6%; outdoor, 41.4%). Chi-square test (χ^2 = 0.208) indicated no significant association (p = 0.381) between eating habit and *H. pylori* status of subjects.

Table 5 shows the logistic regression test indicating the relative risk of each risk factor variable for *H.pylori*. Results indicate that the risk of testing positive for *H.pylori* is significantly greater in subjects aged 15-24 years (OR = 8.0; p = 0.011), 25-34 years (OR = 164; p = 0.006), 35-44 (OR =

4.9; p = 0.038) and 55-64 years (OR = 8.0; p = 0.023) compared to those aged ≥65 years. In contrast, those aged 45-54 years did not indicate significantly greater risk compared to those aged ≥65 years. The females did not indicate significantly (p = 0.546) greater risk for *H.pylori* seropositivity compared to the males. Similarly, no significant greater risk of *H.pylori* were observed in those who make use of filtered (p = 0.673), rain (p = 1.0), borehole (p = 0.237), sachet (p = 0.07) and stream (p = 0.742) water sources compared to those who use 'boiled' water. Furthermore, subjects who eat outdoor did not indicate significantly (p = 0.760) greater risk for *H.pylori* seropositivity compared to those who eat homemade foods.

Table 1. Frequency distribution of subjects according to their age groups and *helicobacter pylori* statuses

AGE GROUPS (years)	HELICOBACTER PYLORI STATUS		Total
	Positive	Negative	
	N (%)	N (%)	
15-24	15 (8.4)	15 (8.4)	30 (16.8)
25-34	41 (22.9)	2 (1.1)	43 (24.0)
35-44	16 (8.9)	26 (14.5)	42 (23.4)
45-54	10 (5.6)	20 (11.2)	30 (16.8)
55-64	8 (4.5)	8 (4.5)	16 (9.0)
≥65	2 (1.1)	16 (8.9)	18 (10.0)
Total	92 (51.4)	87 (48.6)	179 (100)

$\chi^2 = 51.87$; p = 0.000

Table 2. Frequency distribution of subjects according to their sex and *helicobacter pylori* statuses

SEX	HELICOBACTER PYLORI STATUS		Total
	Positive	Negative	
	N (%)	N (%)	
Males	42 (23.5)	35 (19.5)	77 (43)
Females	50 (27.9)	52 (29.1)	102 (57)
Total	92 (51.4)	87 (48.6)	179 (100)

$\chi^2 = 0.536$; p = 0.281

Table 3. Frequency distribution of subjects according to their *helicobacter pylori* statuses and sources of their drinking water

WATER SOURCE	HELICOBACTER PYLORI STATUS		Total
	Positive	Negative	
	N (%)	N (%)	
Filtered	3 (1.7)	4 (2.2)	7 (3.9)
Boiled	6 (3.4)	12 (6.7)	18 (10.1)
Rain	1 (0.6)	2 (1.1)	3 (1.7)
Borehole	16 (8.9)	14 (7.8)	30 (16.7)
Satchet	57 (31.8)	43 (24.0)	100 (55.8)
Stream	9 (5.0)	12 (6.7)	21 (11.7)
Total	92 (51.4)	87 (48.6)	179 (100)

$\chi^2 = 4.86$; p = 0.433

Table 4. Frequency distribution of subjects according to their sex and *helicobacter pylori* statuses

EATING HABIT	HELICOBACTER PYLORI STATUS	Total

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	Positive	Negative	
	N (%)	N (%)	
Homemade	57 (31.9)	51 (28.5)	108 (60.4)
Outdoor	35 919.5)	36 (20.1)	71 (39.6)
Total	92 (51.4)	87 (48.6)	179 (100)

$\chi^2 = 0.208$; p = 0.381

Table 5. Logistic regression indicating the relative risk of each risk factor variable for *H .pylori*

RISK FACTORS	ODDS RATIO (OR)	95% Confidence Interval (CI)	P-value
AGE			
15 – 24	8.0	1.70 – 36.18	0.011
25 – 34	164.0	23.05 – 1169.15	0.000
35 – 44	4.92	1.09 – 21.46	0.038
45 – 54	4.0	0.84 – 18.35	0.167
55 – 64	8.0	1.49 – 40.93	0.023
≥65*	1		
SEX			
Females	0.80	0.44 – 1.45	0.546
Males*	1		
WATER SOURCE			
Filtered	1.50	0.28 – 8.32	0.673
Rain	1.0	0.11 – 9.76	1.00
Borehole	2.28	0.69 – 7.48	0.237
Satchet	2.65	0.95 – 7.38	0.071
Boiled*	1		
EATING HABIT			
Outdoor	0.87	0.48 – 1.58	0.760
Homemade*	1		

*Reference Group

Discussion

The results from this study show that the seroprevalence rate of *H.pylori* in the study population is 51.4%. This is similar to the 58.3% obtained in Ibadan among peptic ulcerative patients[13] and also similar to the 58% obtained in Orlu, Imo state among duodenal and gastric ulcer patients[14]. Similarly, there is a prevalence of 93.3% among peptic ulcerative patients in Kano[15,16] In their epidemiological study in south east Nigeria, it reported a prevalence of 51.75% among those living in high densely populated environment, exposed to faecal contaminated water, poor hygiene and low level of education and 17.66% among those living in low density populated areas. In Kaduna, a study obtained a seroprevalence rate of 80.4% [17]while another got a seroprevalence of 93.6% among dypeptic patients that underwent gastroscopy in Maiduguri[18].

Though the prevalence rate obtained in this study is high, when compared to earlier studies carried out in the Northern part of the country, it is much lower but the values gotten from the eastern and western part of the country are similar to that gotten in this study. This lower prevalence could reflect the comparatively higher standards of hygiene among eastern and western Nigerians compared to that of Northern Nigerians, since *H.pylori* prevalence is higher among those living in high densely populated environment, exposed to faecal contaminated water, poor hygiene and low level of education compare to that of low density populated areas[16].

These findings shows that *H.pylori* is implicated in most peptic ulcer diseases. Studies have shown that use of non-steroidal anti-inflammatory drugs (NSAID) are the major cause of *H.pylori* negative

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peptic ulcer[19]. The seropositivity level increased from 15-24years (8.4%) and peaked at 25-34 years (22.9%) and then declined to 1.1% at greater than 65 years. It was statistically significance with P = 0.000 showing that age is a risk factor for *H.pylori*. The *H.pylori* prevalence according to different age groups as seen in this study is in accordance with what was obtained in other studies, where prevalence of *H.pylori* increased with age at earlier age, then declined in population over 60 years in Pakistan, France and over 50 years in other countries like Vietnam, Algeria and Ivory Coast[20]. In contrast, some studies claimed that *Helicobacter pylori* prevalence increased with age[21]. *H.pylori* infection is acquired at a younger age[3]. In this study also *H.pylori* seropositivity could be seen to be high in younger population which suggests that the infection was acquired during childhood and early adolescence, reaching its peak at adulthood. This observation is in concordance with findings of another study[22]. On the other hand, out of 18 peptic ulcer subjects that fall within the age bracket of 65 years and above, 2(1.1%) were seropositive to *H.pylori* while 16(8.9%) were seronegative to *H.pylori*. Most studies stated that stomach ulcers are more likely to develop in older people[23]. This is because arthritis is prevented by daily use of aspirin and NSAIDs, in addition to age related relaxation of pylorus valve which allows backflow of bile to erode the stomach lining[23]. Also, another study opined that because prostaglandin levels in the gastric mucosa are decreased in elderly patients, ageing is associated with diminished epithelial cell turnover rate and a reduced capacity to repair the gastric mucosa[24]. In this study, there were more females 102(57%) than males 77(43%), and it was observed that *H.pylori* prevalence was more in females. Out of the 92(51.4%) patients that were seropositive, 50(27.9%) were female and 42(23.5%) were male. It was observed that *H.pylori* seropositivity has no significance with sex (p = 0.281) which shows that sex is not a risk factor.

There are varying reports of higher prevalence of *H.pylori* infection in either male or female, but with no significant association between the infectivity rate and sex[25,26] . For instance, a study stated that lifestyle plays a major role in *H.pylori* infection; they also observed that source of water supply used by the participants had an effect on the transmission of infection[16]. Most of their study group used stream water and well water which could be faecally contaminated and few used tap water and bottled water. In this study, though there was no association between source of water and *H.pylori* (p =0.433), majority of the participants that are *H.pylori* positive used satchet water and borehole water which could be contaminated as a result of improper processing of the satchet water, contamination by water vendors or inadequate drilling of the boreholes. Also, eating habits (p = 0.381) did not prove to be a risk factor in this study. This is consistent with the findings of another study[27]. Finally, this research shows that Helicobacter pylori seropositivity was higher in the younger age group which suggests that the infection may have been acquired during childhood. In addition, drinking water sources for majority of the participants who were seropositive for Helicobacter pylori were water packaged in satchets and untreated bore hole water.

Conclusion

Helicobacter pylori is a mjor cause of peptic ulcer in humans. This work has shown that the prevalence of H. pylori seropositivity is high in the study environment but lower than what is obtained in northern Nigeria. H. pylori seropositivity is found to be significantly related to the age of the individual. Moreover, seropositivity was found to increase with age of the subjects, so older adults are at more risk to infections. Since majority of those infected either consumed borehole or satchet water, it suggests that most of the boreholes might be contaminated and that the satchet water may not have been well processed. In addition, even when the satchet water was well processed, people could be infected by the activities and unhygienic attitude of some water vendors.

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