

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

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ABSTRACT

Helicobacter pylori colonize the human gastric epithelium, causing chronic gastritis, peptic ulcer disease and gastric cancer. This work determined the level of IgG seropositivity to *Helicobacter pylori* in peptic ulcerative individuals. A cross sectional study involving 179 peptic ulcerative individuals was conducted. Ethical approval was obtained and informed consent sought. Questionnaire was administered and 5 ml of blood was collected into EDTA containers. Subject selection was done using convenience sampling technique. The *H. pylori* seropositivity was determined using ELISA technique. 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 a significant risk factor for *H. pylori* seropositivity. Females 50 (27.9%) were more seropositive to *H. pylori* than males 42 (23.5%) though the difference was not significant (p=0.281). Moreover, there was no significant relationship between source of drinking water and *H. pylori* seropositivity (p=0.433). Overall, borehole water 16 (8.9%) and sachet water consumers 57 (31.8%) predominated in the seropositive population. The results show that *H. pylori* is high among peptic ulcerative individuals in Nnewi and also, increased levels of interferon gamma may contribute to the development of *H. pylori* associated diseases.

Keywords: *Helicobacter pylori*, Peptic, Risk factors, Seropositivity, Prevalence, Interferon gamma

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 anemia has been shown [7]. However, only 10-15% of those colonized develop disease while 85-90% remains 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,

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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]. Human is the main reservoir of this infection [2,10]. 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, fecal-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].

MATERIALS AND METHODS

Study design

A cross sectional study was conducted among 184 peptic ulcerative individual selected from the medical outpatient clinic and internal medicine clinic of Nnamdi Azikiwe university (NAUTH), Nnewi using convenience 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.

Study population

Subjects included in the study were aged from 15-70 years, having persistent 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 with any sign of peptic ulcer were

excluded. Questionnaires include data on participant's demography, symptoms of peptic ulcer, preferred eating habits, source of drinking water and antibiotics use.

Sample collection

Five (5) milliliters of blood was drawn from the participants using 5 ml syringe and was dispensed into a plain container. The serum was separated and stored at -20°C for Enzyme Linked Immunosorbent Assay (ELISA) *H. pylori* assay. Assay for *H. pylori* IgG antibodies in patients sample was according to the manufacturer's instructions. The ELISA test was performed using Mindray ELISA machine (Shenzhen, China) and the *H. pylori* IgG ELISA kit by Biochem incorporated (Canada).

Purified *H. pylori* antigen was coated on the surface of micro wells. Diluted patients serum was added to the wells and the *H. pylori* IgG-specific antibody, if present, binds to the antigen. All unbound materials were washed away. Enzyme conjugate was added, which binds to the antigen-antibody complex. Excess enzyme conjugate was washed off and substrate and chromogen added. The enzyme conjugate catalytic reaction was stopped at a specific time. The intensity of the color generated was proportional to the amount of IgG-specific antibody in the sample. The results were read using a micro well reader compared in a parallel manner with calibrator and controls [12].

STATISTICAL ANALYSIS

The data was statistically analyzed using SPSS version 20. Values were expressed as mean \pm standard mean error. The student's t-test and chi square were used and considered significant if p-value < 0.05.

RESULTS

The IgG seropositivity for *H. pylori* infection was positive in 92 and negative in 87 subjects, 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*.

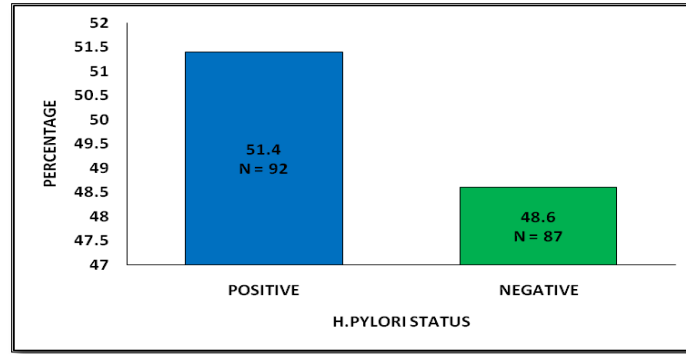


Figure 1. The incidence of seropositivity for *Helicobacter pylori* in peptic ulcer subjects.

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 ($\chi^2=51.87$) shows significant association (p<0.001) between age and *H. pylori* status of subjects.

Table 1. Frequency distribution of subjects according to their age groups and *Helicobacter pylori* status.

Age Groups (Years)	<i>Helicobacter pylori</i> Status		Total
	Positive	Negative	N (%)
	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 reveals the frequency distribution of subjects according to their sex and *Helicobacter pylori* status. 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 ($\chi^2=0.536$) indicated no significant association (p=0.281) between sex and *H. pylori* status of subjects.

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

Sex	<i>Helicobacter Pylori</i> Status		Total
	Positive	Negative	N (%)
	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 shows the frequency distribution of subjects according to their *Helicobacter pylori* status and sources of their drinking water. Results indicate that majority, (57 (62%)) of the seropositive subjects were those who drink ‘sachet’ water, followed by those who consume borehole water (16 (17.4)). Subjects who drink rain water had the least percentage (1.1%) of seropositivity for *H. pylori*.

Subjects who use ‘sachet’ 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 ($\chi^2=4.86$) indicated no significant association ($p=0.433$) between source of water and *H. pylori* status of subjects.

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

Water Sources	<i>Helicobacter pylori</i> Status		Total N (%)
	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)
Sachet	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 shows the frequency distribution of subjects according to their *Helicobacter pylori* status 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 ($\chi^2=0.208$) indicated no significant association ($p=0.381$) between eating habit and *H. pylori* status of subjects.

Table 4. Frequency distribution of subjects according to their *Helicobacter pylori* status and eating habit.

Eating Habit	<i>Helicobacter pylori</i> Status		Total N (%)
	Positive	Negative	
	N (%)	N (%)	
Homemade	57 (31.9)	51 (28.5)	108 (60.4)
Outdoor	35 (19.5)	36 (20.1)	71 (39.6)
Total	92 (51.4)	87 (48.6)	179 (100)

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

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

Risk Factors	Odds Ratio (OR)	95% Confidence Intervals (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 SourceS			
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
Stream	1.50	0.42-5.37	0.742
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 by Abiodun et al. [13] in Ibadan among peptic ulcerative patients and also similar to the 58% obtained by Obiajuru et al. [14] in Orlu, Imo state among duodenal and gastric ulcer patients. Similarly, Tijjani and Umar [15] found a prevalence of 93.3% among peptic ulcerative patients in Kano [16], in their epidemiological study in south east Nigeria, reported a prevalence of 51.75% among those living in high densely populated environment, exposed to fecal contaminated water, poor hygiene and low level of education and 17.66% among those living in low density populated areas. In Kaduna, Nwodo et al. [17] obtained a seroprevalence rate of 80.4% while Olokoba et al. [18] got a seroprevalence of 93.6% among dyspeptic patients that underwent gastroscopy in Maiduguri.

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 South eastern and South western Nigerians compare to that of Northern Nigerians, since *H. pylori* prevalence is higher among those living in high densely populated environment, exposed to fecal contaminated water, poor hygiene and low level of education compare to that of low density populated areas [16]. These findings show that *H. pylori* is implicated in most peptic ulcer diseases. Studies have shown that use of non-steroidal anti-inflammatory drugs (NSAID) is the major cause of *H. pylori* negative 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 group as seen in this study is in accordance with what was obtained in other studies, where prevalence of *H. pylori* increased 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 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 adolescent, reaching its peak at adulthood. This observation is in concordance with findings of Jaff [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, Ananya et al. [24] opined that because prostaglandin levels in the gastric mucosa are decreased in elderly patients, ageing are associated with diminished epithelial cell turnover rate and a reduced capacity to repair the gastric mucosa. In this study, there were more females 102 (57%) than males 77 (43%); 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].

Moreover, Ezugwu and Chibuike [16] stated that lifestyle play a major role in *H. pylori* infection, also they observed that source of water supply used by the participant had an effect on the transmission of infection. Most of their study group used stream water, well water which could be fecally contaminated and few used tap water and bottled water. In this study, though there was no association between source of water and *H. pylori* ($P=0.433$) but majority of the participants that are *H. pylori* positive consume sachet water and borehole water which could be contaminated as a result of improper processing of the sachet water, contamination by water vendors or inadequate drilling of the boreholes. Also eating habit ($P=0.381$) did not prove to be a risk factor in this study. This is consistent with the findings of Valliani et al. [27].

CONCLUSION

Helicobacter pylori are major 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. In addition, the ABO blood group of the individual was also found to be a significant factor in *H. pylori* seropositivity and blood group A are of greater risk. Moreover, seropositivity was found to increase with age of the subjects, so older adults are at more risk of infection. Since majority of those infected either consumed borehole or sachet water, it suggests that most of the boreholes might be contaminated and that the sachet water might not be well processed. In addition even when the sachet water was well processed, people could be infected by the activities and unhygienic attitude of some water vendors. Interferon gamma levels were also found to be higher among those seropositive for *H. pylori* compared to the *H. pylori* seronegative individuals. The gamma interferon contributes to the *H. pylori* associated inflammation which leads to gastric and intestinal ulceration.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interests

AUTHOR'S CONTRIBUTION

C.O.M., C.G.O. J.Z.A, designed research, C.G.O, C.O.M. M.P.O, performed research, A.J.C, M.P.O. analysed data, C.G.O., C.O.M, J.Z.A. wrote paper.

REFERENCES

1. Kanbay M, Gur G, Arslan H (2005) The relationship of ABO blood group, age, gender, smoking and *Helicobacter pylori* infection. *Digest Dis Sci* 50: 1214-1217.
2. Fozieh JM, Tahereh N, Mansour A (2014) Antibacterial activity of garlic (*Allium sativum*) on multidrug resistance *Helicobacter pylori* isolated from gastric biopsies. *Int J Enteric Pathog* 2: 16749.
3. Moayyedi P, Axon AT, Feltbolwer R (2002) Relation of adult lifestyle and socioeconomic factors to the prevalence of *Helicobacter pylori* infection. *Int J Epidemiol* 31: 624-631.
4. Terry K, Williams SM, Connolly L, Ottemann KM (2005) Chemotaxis plays multiple roles during *Helicobacter pylori* animal infection. *Infect Immun* 73: 803-811.
5. Waidner B, Greiner S, Odenbreit S, Kavermann H, Velayudhan J (2002) Essential role of ferritin *Pfr* in *Helicobacter pylori* iron metabolism and gastric colonization. *Infect Immun* 70: 3923-3929.

6. Jing C, Huang ZJ, Duan YQ (2012) Glutathione-S-transferases gene polymorphism in prediction of gastric cancer risk by smoking and *Helicobacter pylori* infection status. *Asian Pac J Cancer Prev* 13: 3325-3328.
7. Celinski K, Kurzeja-Mirosław A, Slomka M (2006) The effects of environmental factors on the prevalence of *Helicobacter pylori* infection in inhabitants of Lublin Province. *Ann Agric Environ Med* 13: 185-191.
8. Zhong C, Li NK, Bi JW (2012) Sodium intake, salt taste and gastric cancer risk according to *Helicobacter pylori* infection, smoking, histological type and tumor site in China. *Asian Pac J Cancer Prev* 13: 2481-2484.
9. Ofonime M, Enobong EI, Emmanuel EE (2012) Seroepidemiology of *Helicobacter pylori* infection among children seen in a tertiary hospital in Uyo, southern Nigeria. *Pan Afr Med J* 12: 39.
10. Lyudmila B (2015) Epidemiology of *Helicobacter pylori* infection. Horizon Scientific Press, p: 278. Available at: <http://www.horizonpress.com/helicobacter>
11. Megraud F, Bessede E, Lehours P (2014) Diagnosis of *Helicobacter pylori* infection. *Helicobacter* 19: 6-10.
12. Ochei J, Kolhatkar A (2000) Medical laboratory science theory and practice. 7th Edn. Tata McGraw-Hill Publishing Company Limited, pp: 356-1174.
13. Abiodun CJ, Jesse AO, Samuel OO, Olayiwola AO, Adegboyega A (2010) Prevalence of *Helicobacter pylori* among Nigerian patients with dyspepsia in Ibadan. *Pan Afr Med J* 6: 18.
14. Obiajuru IOC, Adogu POU (2013) Prevalence of *Helicobacter pylori* and other intestinal parasites amongst duodenal and gastric ulcer patients at Imo state University Teaching Hospital, Orlu, south-eastern Nigeria. *J Med Med Sci* 4: 362-369.
15. Tijjani B, Umar A (2008) Peptic ulcer disease and *Helicobacter pylori* infection at Kano, Nigeria. *Internet J Gastroenterol* 8: 1.
16. Ezugwu RI, Chibuiké C (2014) Epidemiology of *Helicobacter pylori* infection among dyspepsia patients in south-east, Nigeria. *J Pharm Biol Sci* 9: 53-56.
17. Nwodo EN, Yakubu SE, Jatau ED, Yabaya A (2009) Seroprevalence of *Helicobacter pylori* infection in patients with gastritis and peptic ulcer disease in Kaduna, Kaduna state, Nigeria. *Afr J Basic Appl Sci* 1: 123-128.
18. Olokoba AB, Gashau W, Adamu A, Salawu FK (2013) *Helicobacter pylori* infection in Nigeria with dyspepsia. *Ghana Med J* 47: 79-81.
19. Atherton JC, Blaser MJ (2004) *Helicobacter pylori* infections. In: Harrison's Principles of Internal Medicine, ed. Kasper DL, Braunwald E, Fauci AS, Hauser SL, Longo DL, Jameson JL. 16th Edn. New York: McGraw-Hill, pp: 886-889.
20. Ozaydin N, Turkyilmaz SA, Cali S (2013) Prevalence and risk factors of *Helicobacter pylori* in Turkey: A nationally-representative, cross-sectional, screening with the 13C-Urea breath test. *Biometric Central Public Health* 13: 1215.
21. Parente F, Cucino C, Bianchi PG (2003) Treatment options for patients with *Helicobacter pylori* infection resistant to one or more eradication attempts. *Digest Liver Dis* 35: 523-528.
22. Jaff MS (2011) Relation between ABO blood groups and *Helicobacter pylori* infection in symptomatic patients. *Clin Exp Gastroenterol* 4: 221-226.
23. Seyda T, Derya C, Füsün A, Meliha K (2007) The relationship of *Helicobacter pylori* positivity with age, sex and ABO/Rhesus blood groups in patients with gastrointestinal complaints in Turkey. *Helicobacter* 12: 244-250.
24. Ananya C, Sirshendu C, Sandip KB (2012) *H. pylori* induced gastric ulcer: Pathophysiology and herbal remedy. *Int J Biol Med Res* 3: 1461-1465.
25. Lesi OA, Kehinde MO (2003) Prevalence of *Helicobacter pylori* antibodies and predominant symptom complex of patients with dyspepsia. *J Clin Sci* 3: 7-11.
26. Smith SI, Oyedéji KS, Arigbabu AO, Chibututu CC, Atimomo CE, et al. (2001) Seroprevalence of *Helicobacter pylori* infection in patients with gastritis and peptic ulcer in western Nigeria. *Br J Biomed Sci* 58: 97-100.
27. Valliani A, Khan F, Ahmed B (2013) Factors associated with *Helicobacter pylori* infection, results from a developing country, Pakistan. *Asian Pac J Cancer Prev* 14: 53-56.