Prevalence Of *Helicobacter Pylori* And Its Association With ABO Blood Group In Asymptomatic, Ulcer Students Of Western Delta University, Oghara, Nigeria

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Abstract: Epidemiological studies have demonstrated higher frequency of the O blood group and rhesus positive antigen among patients suffering from peptic ulcers. Controversies about the association of the ABO blood group and Rhesus factor in relation to the susceptibility towards infection by this bacillus have been presented. This study aims to determine the involvement of *H. pylori* as a cause of gastritis and peptic ulcer, and also to determine if there is any correlation with ABO blood group in asymptomatic individuals. Blood samples were collected from (fifty) healthy individuals, consisting of 8 males and 42 females aged between 17-30 years (mean 23.5 years). This was accompanied with a questionnaire for demographic data. *H. pylori* one step test kit was used. ABO blood group and rhesus factors were determined by slide agglutination using serum that was serially diluted. Results showed that $64^0/_0$ were sero-positive for *H pylori*. Of the seropositive subjects, $62.5^0/_0$ (n=5/8) were male and 35.7% (n=15/42) were females. The frequency of the ABO and rhesus positive (Rh+) blood groups among seropositive subject was (A=6%, B=8%, AB=4% with rhesus positive 58% and rhesus negative 6%) and among seronegative subjects it was (A=8%, B=0%, AB=0%, 0=28% with rhesus positive 34% and rhesus negative 2%). The results of this study, showed that ABO blood group and rhesus compatibility greatly influenced the seropositivity for *H pylori* infection. Further dilution of the blood samples showed a clear significant rise in titer up to 1:160. The prevalence of cases after the dilution dropped in the following manner (1:20=26%, 1:40=12%, 1:80=8%, and 1:160=2%) the seronegative cases increased during the dilution as (1: 20=74%, 1:40=92%, and 1:160=98%). Prevalence of *H. pylori* infection still remains high after serial dilution. Conclusion: The detection of high prevalence of H. *pylori* infection among asymptomatic individuals and the involvement of ABO blood groups demands that there should be blood screening for every individual especially those that possess the blood groups that are at most risk of infection. [Mordi Raphael Mbadiwe, Braimah Mikalu Rashidat, Igere Bright, N.C. Oyeagwara. **Prevalence Of *Helicobacter Pylori* And Its Association With ABO Blood Group In Asymptomatic, Ulcer Students Of Western Delta University, Oghara, Nigeria.** *N Y Sci J* 2015;8(11):67-75]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork. 10. doi:10.7537/marsnys081115.10.

Key words: Seroprevalence; Asymptomatic individuals; ABO blood group; Helicobacter pylori; Infection.

Introduction

The discovery of *Helicobacter pylori (H. pylori)* by Warren and Marshall, in 1983 was a major breakthrough in the management of dyspepsia (Talley et al., 2005). Numerous studies have reported a high prevalence of *H. pylori* infection among healthy and non-healthy persons in different places (Durazzo et al., 2004; Seyda et al., 2007; Rodrigues et al., 2005). *H. pylori* has been rated as a 'Class One' carcinogen by the World Health Organization (Peterson et al., 2000; Aguemon et al., 2005) It is to peptic ulcer, as cigarette smoking is to lung cancer. Globally, it remains one of the most common infections and it is estimated that 50% of the world's populations are carriers of the bacterium (Goodman et al., 2008).

Helicobacter pylori is a microaerophilic, Gram negative, motile, curved rod and flagellated bacterium with a capability for abundant urease production which has been implicated in several upper gastrointestinal diseases that present as dyspepsia (Suerbaum et al,. 2002; Oluwasola et al,. 2002). It has been suggested that up to 95% of duodenal and 70% of gastric ulcers are attributable to *H. pylori* infection, and most cases occur in middle aged individuals, the highly productive age groups in societies (Rothenbacher 2007). The organism is usually found under the mucus layer in the gastric pit in close opposition to gastric epithelial cells where it causes damages to the cells (Malfertheiner et al, 2007). *H. pylori* infection is recognized as the major cause of chronic gastritis and a factor in the pathogenesis of peptic ulcer disease (Lyra et al,. 2003) and gastric adenocarcinoma (Windsor et al,. 2005). *H. pylori* infects a large portion of the world population (Windsor et al., 2005), but there are large differences in the prevalence of infection among ethnic groups (Santos et al., 2005; Pillary et al., 2007). Infection occurs early (Santos et al., 2005) and *H. pylori* sero-positivity increased with age (Robertson et al., 2003; Naja et al.. 2007). Lower socioeconomic status

(crowded living conditions) is associated with high infection rates (Seyda et al., 2007; Naja et al., 2007). The pylori infection may, in some instances, be a zoonosis (Dore et al., 1999). Once *H. pylori* infection is acquired, it persists for decades and probably for life in untreated persons (Pillay et al., 2007). Education level was associated with negative *H. pylori* status (Shi et al., 2008). The association of ABO blood groups with some infectious and non-infectious diseases has been described (Jaff MS 2011). Previous serological studies have related a higher prevalence of antibodies against *H. pylori* in some professions (abattoir workers, shepherds and veterinary workers) to direct contact with *H. pylori* infected animals (Papiez et al., 2003). Also, *H. pylori* has been isolated from the intestinal tract of dogs, cats and sheep (Dore et al., 2001). Research has also demonstrated that *H. pylori* can live for several days in milk and water in its infectious bacillary form and in river water for several months in a non-culturable but viable form (Braganca et al., 2007). *Helicobacter pylori* was identified as the main etiology of peptic ulcers, chronic gastritis, and a variety of gastrointestinal symptoms. Many epidemiologic studies had found that non secretors of ABO blood group antigens and individuals of blood group O were overrepresented among patients with peptic ulcers, (Rosenstock et al., 1997). These studies encouraged many researches to investigate the relationship between ABO blood groups and their secretor status with peptic ulcer. Many authors reported an association between blood group O and *H. pylori* infection (Kanbay et al., 2005), while others failed to find such an association. (Niv et al., 1996). Peptic ulcer disease is now viewed as an infectious disease since eradication of *H. pylori* leads to its cure. (Malfertheiner et al., 2007). The paucity of information in Nigeria as regards the relationship of ABO blood group and rhesus compatibility to *Helicobacter pylori* prevalence and the need to screen for *H. pylori* antigen in blood samples of asymptomatic individuals necessitated this study.

Materials And Methods

This study which was prospective and cross-sectional was conducted in Western Delta University, Oghara, Delta State. A total of fifty volunteers were recruited for the studyand this consists of eight (8) males and fourty two (42) females. Demographic data was obtained by means of standard questionnaire. A questionnaire is a good tool that is employed in various aspects of epidemiological studies to gather information. In this study, a questionnaire was administered to the subjects in order to obtain their socio-demographic and economic data. Data such as age, sex, socio economic status, history of any peptic ulcer disease, and drug taken. Blood samples were collected in duplicate from each voluntary participant using 5mls syringes. 2.5mls each was dispensed into a sterile anti-coagulated bottle containing Ethylene Diamine Tetra-acetic acid (EDTA). The other 2.5mls was dispensed into a sterile coagulated bottle containing coated beads which is the clothing factor. This was spun using the centrifuge and serum was separated into a sterile plane bottle and this was stored in the fridge at 2-8oC. Based on the presence of the antigen in the blood samples, the samples were further serially diluted in a four-fold dilution. The other part of the samples was used to carry out ABO blood group or Landsteiner group. Sterile anti-coagulated Ethylene Diamine Tetra acetic acid (EDTA) bottles, sterile coagulated bottle, syringes, cotton wool, 70% alcohol, *H. pylori* global test kit, pipettes, hand gloves, Face mask, test-tubes, test-tube rack, autoclave, aluminum foil paper, normal saline, blood samples, thermo-cool refrigerator. All test-tube and beakers used in this study were thoroughly washed with detergent and jik then rinsed in clean water to ensure that they were grease-free and were covered with cotton wool and aluminum in an autoclave at $121^{O}C$ for 15 min. at 15 pound/pressure.

Selection Of Subjects

In selecting these subjects, the aim of this study was explained to them and they conceded to continuing with the test. Samples were collected from eligible members based on the questionnaire data. Those with history of peptic ulcer and those currently using anti-ulcer medications were excluded from the study. Subjects' participation in this study was voluntary. Information collected from participant was kept confidential.

Detection Of *Helicobacter Pylori* Antibodies Using Blood Serum On Global Kit

The *H. pylori* one step test device utilizes a combination of *H. pylori* antigen coated particles and anti-human IgG to qualitatively and selectively detect *H.pylori* antibodies in serum just within ten minutes. The blood samples were allowed to cloth thereafter, they were spun using the bucket centrifuge at 4000 revolution per minute (RPM) for five (5) minutes. The global kit was removed from the foil pouch and placed on a sterile laboratory bench. Using the disposable specimen dropper, three drops (apporximatelly 100nl) of the serum was dispensed on the specimen well labeled **(S)** on the test kit. This was allowed to flow and result was read after 10 miuntes. Interpretation of results was based on the position and number of line appearing on the test kit. Positive results were read as having two distinct red lines, one red line on the control region (C) and another line in the test region (T). On the other hand the negative result was read as having one red line only on the control region (C) and no apparent red line in the test region (T).

Preparation Of Slide Agglutination ABO Blood Grouping Landsteiner Blood Group And Rhesus Compactibility

According to the method used by Landsteiner and Wiener, ABO and Rhesus (Rh) blood groups were determined for both sero-positive and sero-negative subjects, using standardized haemaglutination methods that is slide agglutination test. First the blood samples collected in a sterile anti-coagulated ethylene diamine tetra acetic acid (EDTA), a drop of each blood sample, was placed on each part of the rocking tile. Equal drops of anti-sera A, B, and D were placed on each section of the tile. Using sterile glass rods, it was used to mix each and was in turn cleaned. The rocking tile was rocked back and forth for two (2) minutes in other to detect the antibodies. Thereafter the results were interpreted as follows:

- An agglutination in A and O indicates A rhesus "D" positive
- An agglutination in B and O indicates B rhesus "D" positive
- An agglutination in A,B and O indicates AB rhesus "D" positive
- An agglutination in O only indicate O rhesus "D" positive
- An agglutination in A and non in O indicate A rhesus "D" negative
- An agglutination in A, B and non in O indicates AB rhesus "D" negative
- There was no agglutination in all indicates O rhesus "D" negative

The results with the sero-positive and sero-negative were compared with the blood group and Rhesus factor

Four-Fold Serial Dilution For *H. Pylori* Antibody Detection

Serial dilution means diluting with a constant factor. Blood samples collected were spun using the bucket centrifuge at 4000 revolution per minute (RPM) for five minutes. Thereafter, the serums were separated using pasture pipettes. They were stored in the fridge at -20^0C. During serial dilution, the sera were brought out and allowed to completely thaw to room temperature that is 20^0C. The serums were further serially diluted in a four-fold dilution in other to know the highest titre that is highest dilution in which the reaction stopped. First normal saline was prepared by weighing 0.85g of sodium chloride and dissolved into 1000ml of serial water. Six test tubes were arranged on the test tube rack and were labeled one to six respectively. 1ml of the serum was dispensed into first tube this serves as the neat, (1:10 dilution) 0.5ml of normal saline was dispensed into the remaining 5 tubes with 5ml pipettes. Thereafter, using a 1ml pipette, 0.5ml of serum was transferred from tube 1 to tube 2. The content was properly mixed by carefully drawing the liquid up into the pipette and discharging it slowly back down into the tube three times. This process was repeated serially to tube five by transferring 0.5mls and the last 0.5mls was discarded instead of adding to tube six. Thus tube six has only normal saline which was used as the negative control. The *H. pylori* one step test kit contains H. pylori antigen coated particles and anti-human IgG coated on the membrane. It was allowed to flow and results were interpreted after 10 minutes. For serum which contains *H. pylori* antibodies a red colored line appeared on the test region (T) and also on the control region (C) of the test kit, this was reported as positive. While the serum which does not contain *H. pylori* antibodies, a red colored line appeared only on the control region (C) of the test kit, this was reported as negative. This was based on manufacturer's recommendation. The antibody titer was recorded as 1:20, 1:40, 1:80, 1:160, and 1:320.

The results of this study were compared with sero-positive and sero-negative subjects as well as ABO or Landsteiner blood group and rhesus compatibility.

Statistical Analysis

Data generated from this study were analyzed using Statistical Package for Social Sciences (SPSS) Chicago IL. Chi-square test was used to detect statistically significant differences among variables. P values < 0.05 were considered significant. Chi-square test was used for the analysis of the association of *H. pylori* infection according to ABO blood group and rhesus factor. The results were recorded based on percentages.

Results

4.1.0 Prevalence Of *H. Pylori* And Its Association With ABO Blood Group And Rhesus Compactability.

Of the 50 blood samples analyzed, it was found that 32 samples were positive and 18 were negative.

Pylori antigen was detected in 32 subjects, yielding an overall prevalence of 64% while the sero positive as compared to other ABO blood types (Tables 2). In the same vain rhesus "D" positive also has the highest *H. pylori* sero positive (3). The prevalence was higher in females than in males. Of the 42 females used during the study, *H. pylori* was detected in 27 (64.3%) as against 5 (62.5%) of 8 males using serum on global test kit (Table 4). There was a reduced detection of *H. pylori* antigen in a four-fold serial dilution amongst the 27 (64.3%) females of 42 females, 11 (26.2%) were positive after serial dilution, whereas amongst the 8 males, only 2 (25%) of the males where positive. It was also observed that H.

pylori prevalence is higher amongst blood group O   and rhesus "D" positive individuals (Table 2 and 3).

Table 1: Frequency of occurrence of H. pylori antigen detection.

H. pylori samples	Total number (N)	Percentages (%)
H. pylori positive	32	64
H. pylori negative	18	36
Overall total	50	100

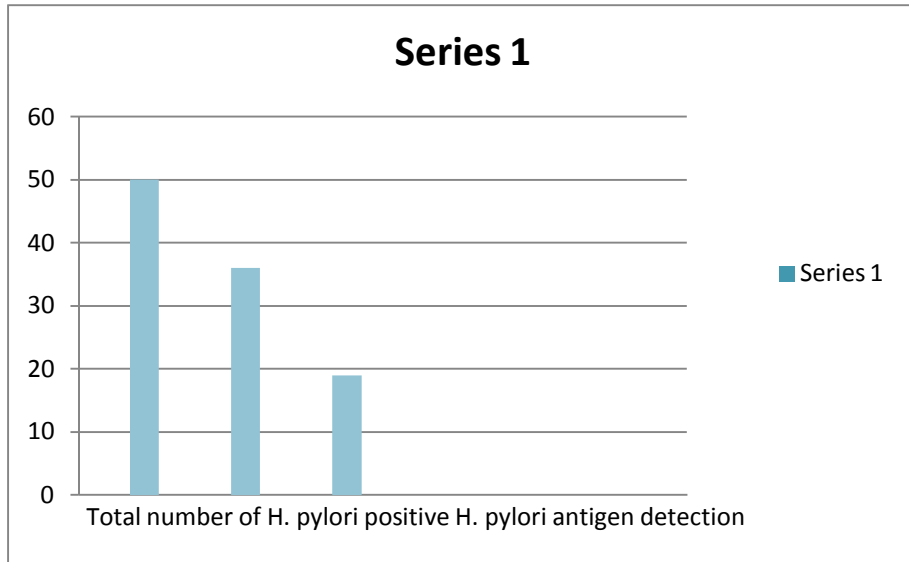**Figure 1: frequency of occurrence of H. pylori antigen detection**

Table 2: Association between H. pylori and ABO blood group

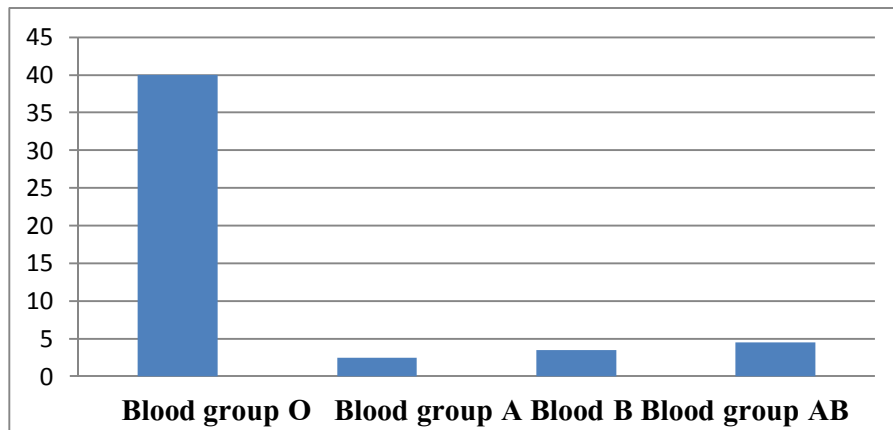|	Percentage occurrence in blood group							
Antigen in Serum	Total number	A		B		AB		O	
		Number	(%)	N	(%)	N	(%)	N	(%)
H. pylori positive	32	3	6	4	8	2	4	23	46
H. pylori negative	18	4	8	0	0	0	0	14	28

Figure 2: Percentage frequency distribution of H. pylori amongst ABO blood group

Table 3: Association between *H. pylori* and Rhesus compatibility

Samples	Total Number (N)	Percentages (%)	Rhesus factor			
			Rhesus "D" positive		Rhesus "D" negative	
			(N)	(%)	(N)	(%)
H. pylori Positive	32	64	29	58	3	6
H. pylori Negative	18	36	17	34	1	2
Overall Total	50	100	46	92	4	8

Table 4: Frequency of occurrence of *H. pylori* amongst typed gender

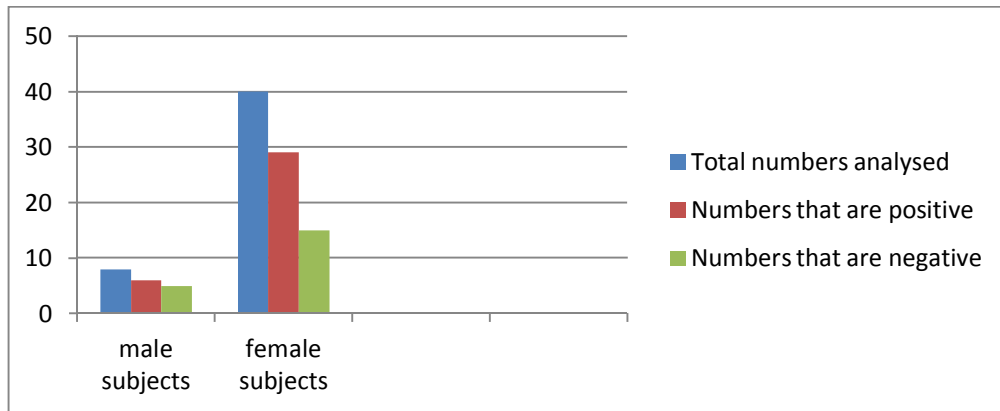| Samples	Males		Females	
	(N)	(%)	(N)	(%)
H. pylori positive	5	62.5	27	64.3
H. pylori Negative	3	37.5	15	35.7
Overall total	8	100	42	100

Figure 3: frequency of *H. pylori* antigen detection according to gender

Table 5: Association between *H. pylori* and rhesus compatibility

Samples	Rhesus "D" positive				Rhesus "D" negative			
	O	A	B	AB	O	A	B	AB
H. Pylori positive	21 (60%)	3(50%)	4 (100%)	1 (100%)	1 (100%)	0	0	1 (100%)
H. pylori negative	14 (40%)	3(50%)	0	0	0	1 (100%)	-(0)	0

TABLE 6: Association between *H. pylori* and female ABO blood group with rhesus compatibility

Samples	ABO blood group (%)				Rhesus "D" positive (%)				Rhesus "D" negative (%)			
	A	B	AB	O	A	B	AB	O	A	B	AB	O
H. pylori positive	1(2.4)	4(9.5)	2(4.8)	20(47.7)	1(2.4)	4(9.5)	1(2.4)	18(42.9)	0	0	1 (2.4)	2(4.8)
H. pylori negative	2(4.8)	0	0	12(28.6)	2(4.8)	0	0	12(28.0)	1(2.4)	0	0	0

TABLE 7: Association between *H. pylori* and male ABO blood group with rhesus compatibility

Samples	ABO blood group				Rhesus "D" positive				Rhesus "D" negative			
	A	B	AB	O	A	B	AB	O	A	B	AB	O
H. pylori positive	2(25)	0	0	3(37.5)	2(25)	0	0	3(37.5)	0	0	0	0
H. pylori negative	1(12.5)	0	0	2(25)	1(12.5)	0	0	2(25)	0	0	0	0

4.2.0 Serial Dilution Of Samples

The blood samples were further diluted and test carried out to detect possible positive blood cases in the same comparative study. The results are as shown in the tables below. It is of interest to note that there were clear significant variations between the undiluted and diluted blood samples based on the rise in titre. After a four-fold dilution, the titre was still high up to 1:160.

As the positive cases kept reducing, the negative cases kept increasing according to the titres (Table 1) while figure 1 shows the variations of titre. Blood group O rhesus "D" still has the highest number of *H. pylori* infection which means that they are more prone to *H. pylori* infection (Table 4 and 5) there was a reduction of antibody titre of males subjects in Table 6 while that of females was still high (Table 7).

TABLE 8: A four-fold serial dilution frequency of occurrence *H. pylori* antigen detection

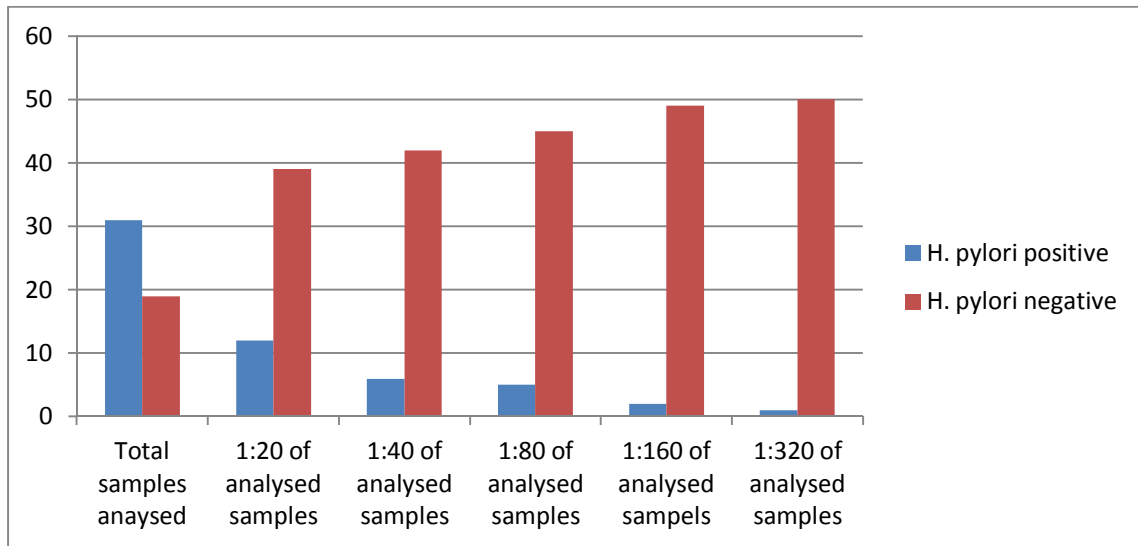| samples	Serum neat	1.20	1.40	1.80	1.160	1.320
H. pylori positive	32	13(26%)	6(12%)	4(8%)	1(2%)	0
H. pylori negative	18	37(74%)	44(88%)	46(92%)	49(98%)	50(100%)
Overall total	50	50 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)

Figure 4: A four-fold serial dilution showing frequency of occurrence of *H. pylori* antigen detection

TABLE 9: *H. pylori* positive antigen detection in relation to ABO blood group after a four-fold serial dilution.

ABO blood group	1:20	1:40	1:80	1:160	1:320
A	2	1			0
B	3	2	1		0
AB	1				0
O	7	3	3	1	0

TABLE 10: *H. pylori* positive antigen detection in relation to rhesus compatibility after a four-fold serial dilution.

	Rhesus	1:20	1:40	1:80	1:160	1:320
Rhesus "D" positive	A	2	1			0
	B	3	2	1		0
	AB	1				0
	O	5	3	3	1	0
Rhesus "D" negative	A					
	B					
	AB					
	O	2				

TABLE 11: A four-fold serial dilution for the detection of *H. pylori* antigen amongst male subjects

Samples	Serum neat	1:20	1:40	1:80	1:160	1:320
H. *Pylori* positive	5	2(25%)	1(12.5%)	0	0	0
H. pylori negative	3	6(75%)	7(87.5%)	8(100%)	8(100%)	8(100%)
Overall total	8	8(100%)	8(100%)	8(100%)	8(100%)	8(100%)

TABLE 12: A four-fold serial dilution for the detection of *H. pylori* antigen amongst male subjects

Samples	Serum neat	1:20	1:40	1:80	1:160	1:320
H. *Pylori* positive	27	11(26.2%)	5(11.9%)	2(4.8%)	1(2.4%)	0
H. pylori negative	15	31(73.8%)	37(88.1%)	40(95.2%)	41(97.6%)	42(100%)
Overall total	42	42(100%)	42(100%)	42(100%)	42(100%)	42(100%)

Discussion

Helicobacter pylori infection has a relevant clinical importance because individuals infected with *H. pylori* develop serum antibodies which correlate strongly with histologically confirmed *H. pylori* infection and the testing for *H. pylori* antibodies helps in early detection of "silent" peptic ulcer (Vaira et al., 1994). This study showed that amongst the fifty asymptomatic subjects analyzed, 32(64%) were positive which indicates that these subjects were carriers of the organism (H. pylori) without knowing that they are already infected (silent sufferers) as previously reported by Vaira et al., 1994. Reports have also indicated substantial evidence for the acquisition of H. pylori primary infection at early age, both in developed and developing countries (Ndip et al., 2004; Alborzia et al., 2006; Ahmed et al., 2007). The mode of H. pylori transmission however remains controversial. Results of this study also showed a high percentage association between the O blood group and infection caused by *H. pylori* (percentage = 64%), a finding which is consistent with literature reports (Kanbay et al., 2005, lin et al., 1998). In this study, the prevalence of sero-positive H. pylori infection was 64% (32 of 50) even after serially diluting the blood serum, the titre still remains very high (up to 1:160) in asymptomatic students in Western Delta University, Oghara, Delta State, Nigeria is higher than the average prevalence in the world's population (50%) as reported by Parsonnet 2006. This made the second hypothesis of this study a valid hypothesis. This present study is in view that the higher susceptibility of O blood group individuals to *H. pylori* infection is most probably due to the higher frequency of secretor status in O blood group individuals (Jaff 2010). This view is supported by a previous demonstration, by Alkout et al, (2000) that H antigen represents an important receptor expressed in the gastro-duodenal mucosal cells to which *H. pylori* adheres, (Alkout et al., 2000) which also enhances colonization of *H. pylori* bacteria.

It was also observed from the administered questionnaire that most of the subjects who expressed high pathogenic response were of the blood group O. This observation was previously reported that Blood group O individuals expressed a higher inflammatory response of *H. pylori* with higher levels of lymphocyte infiltration in the gastrointestinal mucusa, (Alkout et al., 2000; Abdulhamid et al., 2000), a lower level of Von Willebrand's factor, (Franchini et al.,2007; Brown et al., 2003) and a higher frequency of secretor status; (Jaff 2010), all these together, in the view of the present author, explain these individuals' increased susceptibility to peptic ulceration.

Regarding rhesus status, this study showed a relative difference between the sero-positive subjects and sero-negative subjects as the rhesus seropositive subjects had a high prevalence than the rhesus seronegative subjects, although this report did not agree with the general population as previously reported by the studies of Petrovic et al., 2011.

In this study, *H. pylori* colonization was higher in females than in males with seropositive prevalence of females (64:3%) and males (62.5%) as seen in

some other studies reported (Kanbay et al., 2005; Lacy et al., 2001). This result is not in support with the finding of previous reports (Ndip et al., 2004), in which a higher prevalence rate was reported in males than in females. Kaltenthaler et al., (1995) reported that H. pylori infection is generally higher in males than in females and suggested that this might relate to young boys having poorer hygiene than young girls because infection is acquired at an early age. While some other studies have not noticed such relations to gender (Petrovic et al., 2011; Seyda et al., 2007; Khan et al., 2007; Alazmi et. el., 2010; Farshad et al., 2010). We therefore think that our divergent observation in this study could be due to the different sample sizes used because more females participated than males.

From various studies (including this one), genetic predisposition, as well as environmental factors, are suggested as important factors influencing *H. pylori* infection, a view supported by the Malaty and colleagues study on twins (Malaty et al., 1998).

Conclusion

The results obtained from this study have revealed a high prevalence of *H. pylori* antigen from blood specimen of asymptomatic individuals and also a correlation between *H. pylori,* peptic ulcer and gastritis. There is need to improve sanitation, socio-economic standard of living and purified water supply. There should be measures to protect those at most risk of infection especially O blood group individuals who are more susceptible to *H. pylori* infection and/or they have more cellular and immunological response to it (expressed by sero-positivity) than other ABO blood groups.

Acknowledgment

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