Seroprevalence of *Helicobacter pylori* Infection among Pregnant Women Attending Antenatal Clinic at Yusuf Dantsoho Memorial Hospital, Kaduna

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Abstract: *Helicobacter pylori* is a Gram-negative bacillus known to colonize the stomach and plays a role in the creation of multiple gastrointestinal disorders which causes considerable morbidity and impose a major burden upon healthcare particularly in developing countries. This study was conducted to determine the seroprevalence of *Helicobacter pylori* among pregnant women attending antenatal clinic at Yusuf Dantsoho Memorial Hospital, Kaduna State Nigeria. Hundred (100) blood samples were collected from pregnant women and screened for the presence of *Helicobacter pylori* antibodies using NOVA TEST One Step Diagnostic Rapid Test Cassette. Questionnaires were also used to determine the risk factors associated with *H. pylori* infection. The results obtained showed an overall prevalence of 29%. The results of prevalence by age group showed that age group greater than 38 years had the highest prevalence of 51.7%, while age group 18 – 22 had the lowest prevalence of 6.9%. Statistical analysis of the risk factors associated with *H. pylori* infection using chi-square showed no significant association (p-value greater than 0.05) between *H. pylori* infection with pregnancy stages in trimesters, gastrointestinal illness, heartburn, and source of water for drinking. However, this study showed significant association (p-value of 0.03) between *Helicobacter pylori* infection and hyperemesis gravidarum among the study population. Therefore, there is need to create awareness of *Helicobacter pylori* infection for its possible prevention and routine screening for *Helicobacter pylori* be implemented for all antenatal women in the hospital.
Keywords: *Helicobacter pylori,* Pregnancy, pregnant women, seroprevalence

INTRODUCTION

Helicobacter pylori is a Gram-negative bacillus known to colonize the stomach and plays a role in the creation of multiple gastrointestinal disorders which is the most common chronic infection around the world (Wayermann *et al.,* 2016). Its presence is associated with gastritis; substantial evidence indicates that it causes peptic ulcers, duodenal ulcer, chronic gastritis and it is also involved in the development of gastric cancer, type B low-grade mucosa-associated lymphoid tissue lymphoma, and cardiovascular disease (Labignet *et al.,* 2009; Kanbay *et al.,* 2011; Aslan *et al.,* 2013). Moreover, infection with *H. pylori* is associated with conditions such as atherosclerosis, insulin resistance, diabetes mellitus and some autoimmune disease (Ajose and Naito, 2009). Pregnancy is a physiological condition associated with both anatomical and biochemical alterations all geared towards the sustenance of the growing foetus (Ajose and Naito, 2009). *Helicobacter pylori* infection in pregnancy is also the primary causes of iron deficient anaemia, malformation, miscarriage and growth restriction (Brenner *et al.,* 2016).

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These pregnancies related disorders are potentially life threatening infections for both mother and fetus (Brenner *et al.,* 2016). Since *H. pylori* infection is most likely acquired before pregnancy, it is widely believed that hormonal and immunological changes occurring during pregnancy could activate latent *H. pylori* infection with a negative impact not only on maternal health (nutritional deficiency, organ injury, death), but also on the fetus (insufficient growth, malformation, death) and sometime consequences can be observed later in life (Wayermann *et al.,* 2016).

In the past, tests to identify *Helicobacter pylori* in the diagnoses of infection were not usually carried out as most of the infections like ulcer and gastritis were not previously believed to have a microbial cause (Kanbay *et al.,* 2011).

Hence, information on the prevalence of *Helicobacter pylori* at local level are not well documented (Wayermann *et al.,* 2016). For this reason, the study was designed to provide baseline pre-control data required for planning in order to provide possible solutions to the problem.

This study may help to throw more light on the prevalence of this infection among pregnant women through the identification of the presence of *Helicobacter pylori* antibodies in blood samples.

MATERIALS AND METHODS

The following were the materials used: *H. pylori* NOVA TEST One Step Diagnostic Rapid Test Cassette, Pasteur pipette, Plain bottles, Ethylene diaminetetracetic acid (EDTA) bottles, Centrifuge machine, Syringes, Tap water, Disinfectant, Tourniquet, Needles, Medical hand gloves, Masking tape, Permanent marker, Conical flask, Blood bottle rack, Face mask and Cotton wool.

Study Design and Population

The study was a hospital based cross sectional study which span through a period of four months. The study was conducted at Yusuf Dantsoho Memorial Hospital Tudun Wada, Kaduna. The participants were pregnant women attending antenatal clinic during the period of the study.

Sample Size

The Sample size was determined using the formula below (Kitagawa *et al.,* 2008).

$N= Z^2 pq/d^2$

Where; N= Required Sample size, Z= Standard normal distribution at 95% Confidence interval = 1.96 p= reported prevalence rate 7% = 0.07, d= Allowable or permissible error, q= 1 – p = 1-0.07 =0.93 N=? p= 0. 07 d= 0.05

$$N = \frac{1.96^2 \times 0.07 \times 0.93}{(0.05)^2} = \frac{3.8416 \times 0.07 \times 0.93}{0.0025} = \frac{0.2500}{0.0025}$$

N= 100 Samples.

Data Collection and Processing

Demographic Characteristics and Exposure to Risk Factors

Before enrollment, informed consent of the participants were sought and obtained.

Questionnaires were administered through interview to each participant to obtain information associated with certain risk factors; age group, pregnancy stages in trimester, source of water for drinking, history of gastrointestinal illness and symptoms like hyperemesis gravidarum and heartburn.

Specimen Collection and Storage

A total of 100 blood samples were collected strictly following standard operational procedure from pregnant women attending ante-natal clinic at Yusuf Dantsoho memorial Hospital Tudun Wada, Kaduna.

With the aid of a well-trained laboratory technologist (phlebotomist), the whole blood was drawn by using a 2ml syringe. About 2ml of venous blood was drawn from each pregnant woman aseptically by venipuncture and then dispensed into a sterile labeled anticoagulant free container (EDTA) bottle for the detection of *H. pylori* antibodies, the blood samples were transported to Kaduna State University, Microbiology laboratory.

The blood samples were processed by centrifugation at 5000 rpm for 3 minutes to separate the serum samples from the blood. Using micropipette, the serum were drawn from EDTA bottle and then transferred into plain bottles. All the bottles were labeled properly and serum samples were refrigerated until the analysis for antibody to *Helicobacter pylori* was carried out (Kitagawa *et al.,* 2008).

Assay for Detection of *Helicobacter pylori* Antibodies using NOVA TEST One Step Diagnostic Rapid Test Cassette

At the laboratory, the work bench was cleaned with sterile cotton wool and methylated sprit. Gloves were worn and used to take the blood sample bottles out of the refrigerator. The serum bottles were properly arranged on a blood bottle rack and allowed to regain room temperature and each of the *H. pylori* test cassette was taken out of its foil pouch and placed on the flat, dried disinfected surface. A micro litre pipette was used to obtain the serum sample which was dropped in the sample well of the test cassette. The red color moved across the window in the centre of the test device to show that the device was working (Cardaropoli, 2014). The left section of the result window is the "Control line" the right section of the result window is the "Test line.

The results were interpreted within 10 minutes at room temperature as positive, negative and invalid result respectively.

Positive Result: The presence of two red color lines within C and T section of the window no matter which band appears first, indicated the presence of *H. pylori* antibody in the specimen. The result was read positive

Negative Result: Presence of the red color line only within the C band indicated that no detectable *H. pylori* antibody was present in the specimen. The result was red negative.

Invalid: Absence of the red color line within C band, the assay were invalid regardless of any line that appeared on the T band (Cardaropoli, 2014).

Data Analysis and Interpretation

The data from the study was analyzed using excel, Chi-square method. P-values less than 0.05 were considered significant. Results were expressed using frequency and percentages. Figure and tables were used for data presentation.

RESULTS

Out of 100 samples analyzed, 29% were positive for *H. pylori* while 71% were negative for *H. pylori* infection (Figure 1). The analysis of the results obtained by age group (Table 1) revealed that age group >38 had the highest prevalence of 51.7% and age group 18-22 had the lowest prevalence of 6.9% in the study population. Table 2 showed the relationship between *H. pylori* infection and stages in pregnancy. The highest prevalence of *H. pylori* infection (68.9%) were seen during the first trimester. However, the prevalence declined continuously as women entered the third stage (trimester) of pregnancy with the least prevalence (10.3%) of *Helicobacter pylori* infection. The data analysis X^2 =4.032, df= 2, P-value = 0.583) showed no relationship between *Helicobacter pylori* infection and the stages of pregnancy (in trimesters) among the study population (Table 2). Table 3: is the results of the prevalence rate of *H. pylori* with associated risk factors. From the results, pregnant women with history of gastrointestinal illness have the highest prevalence (65.5%) and those pregnant women without the history of gastrointestinal illness have the prevalence of (34.5%). The data analysis (X^2 =3.824, df =1, p-value =1.084) showed no association between *H. pylori* and history of the gastro intestinal illness in the study population. The relationship between *H. pylori* and sources of drinking water as shown in Table 3 revealed that well water users are at higher risk of acquiring the organism with total percentage of 44.8% while tap water had 31% and borehole water had lowest percentage of 24%. The data analysis (X^2 =2.348, df =2, p-value = 0.282) showed no association between *Helicobacter pylori* infection and source of drinking water in the study population. The prevalence of *H. pylori* in relation to Hyperemesis gravidarum in the study (Table 3) showed that, 72.4% of the subjects that were positive for *H. pylori* infection were pregnant women with history of Hyperemesis gravidarum, while 27.6 % did not experience hyperemesis gravidarum. Data analysis (X^2 =3.213, df = 1, p-value = 0.030) showed significant relationship between *H.* pylori infection with hyperemesis gravidarum in the study population. The result of the relationship between *H. pylori* infection and heartburn (Table 3) showed that 41.4% of pregnant women who experienced heartburn were positive to *Helicobacter pylori*, while 58.6% of pregnant women who did not experience heartburn in their pregnancy were also positive to *Helicobacter pylori* infection. Data analysis (X^2 =2.35, df = 1, p-value = 0.142) revealed no association between *Helicobacter pylori* infection and heartburn in the study population.

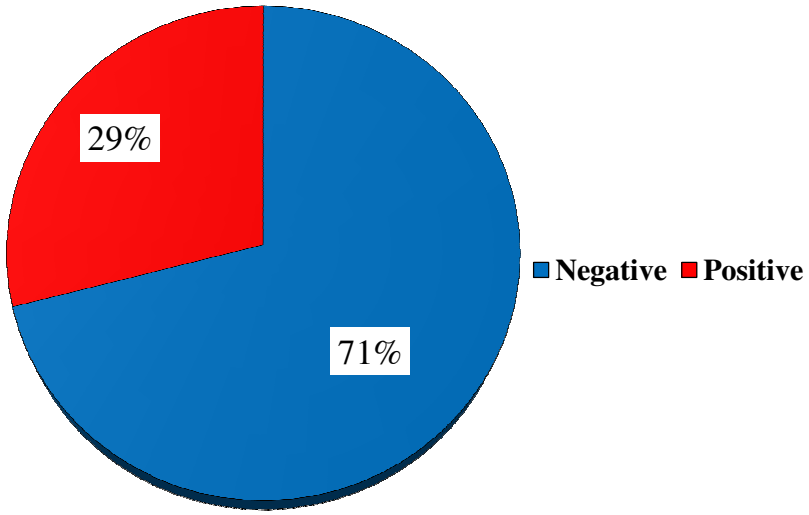

Figure 1: Prevalence of *H. pylori* infection amongst pregnant women in the study population

Table 1: Prevalence of *H. pylori* by age group

Age group (years)	No. screened	No. positive	Percentage %
18 – 22	22	2	6.9
23-27	20	3	10.3
28-32	16	3	10.3
33-37	16	6	20.8
>38	26	15	51.7
Total	**100**	**29**	**100**

Table 2: Prevalence of *Helicobacter pylori* by stages in pregnancy

Trimester Stages	No. examined	No. Positive	Percentage %
First trimester	41	20	68.9
Second trimester	24	6	20.7
Third trimester	35	3	10.3
Total	**100**	**29**	**100**

Table 3: Prevalence of *H. pylori* and associated risk factors

Risk factors	No. examined	No. Positive	Percentage %	Total
Gastro intestinal illness				
Pregnant women with history of gastrointestinal illness	23	19	65.5	**100**
Pregnant women without history of gastrointestinal illness	48	10	34.5	
Sources of drinking water				
Tap water	27	9	31.03	
Borehole water	29	7	24.1	**100**
Well water	15	13	44.8	
History of Hyperemis gravidarum				
Pregnant women with hyperemesis gravidarum	33	21	72.4	
Pregnant women without hyperemesis gravidarum	38	8	27.6	**100**
Heart burn				
Pregnant women with heart burn	39	12	41.4	**100**
Pregnant women without heart burn	32	17	58.6	

DISCUSSION

The overall seroprevalence of 29% *H. pylori* infection was found among the study population. This result is consistent with the findings of Ndububa *et al.* (2014) and Kure *et al.,* (2012) who reported prevalence rate of 30% and 28.9% respectively, in a study conducted to determine the seroprevalence of *H. pylori* antibodies. However, the result of this study does not agree with the work of Nwodo, (2012) in a study conducted in South Eastern part of Nigeria, who reported higher prevalence of 70% of *H. pylori* infection. The reason for the differences in prevalence could be due to the sample size, socioeconomic status and poor personal hygiene as the organisms tend to thrive more in a low socio economic and dirty environment (Dogara *et al.,* 2010).

The prevalence of *H. pylori* infection by age group were observed, age group greater than 38 had the highest prevalence of 51.7% of *H. pylori* infection. This results agree with the work of Abubakar *et al.* (2013) who observed 50.5% of *H. pylori*, in a study conducted to determine the prevalence of *Helicobacter pylori* infection and associated risk factors. The reason could be due to old age as older people are prone to various infectious agents because as people age their immune system and other organs become very weak to fight against some diseases.

The relationship between *H. pylori* infection and pregnancy stages were investigated, 68.9% of women at first trimester stage were positive to *H. pylori* infection. This finding is similar to the work conducted in Kano, North Western part of Nigeria by Alhassan *et al*., (2009) who reported high significant correlation between pregnancy and *H. pylori* infection with the prevalence rate of 69%. The reason for the correlation is not far-fetched, this is may be as the result of the increased accumulation of the body fluid caused by elevated steroid hormones in pregnant women, where shift in pH may occur (this includes the pH of the gastrointestinal tract) which can result in the manifestation of a subclinical *H. pylori* infection, which can exacerbate gastrointestinal symptoms (Yahaya *et al.,* 2009).

The relationship between gastrointestinal illness and *H. pylori* infection were investigated as a risk factor, the result revealed that 55% of pregnant women with history of gastrointestinal illness were positive to *H. pylori* infection. This result is comparable with the study conducted in Gombe State by Imran *et al.,* (2016) who reported prevalence rate of 56.3%. This might be because as the bacteria infect the stomach wall, it controls the secretion of stomach acid leading to overproduction of hydrochloric acid, paving the way for ulceration and the acid producing cells themselves are affected and less acid is secreted, causing hypochlorhydria or low stomach acid. The consequences of low stomach acid, is production of low vitamin B12, since it is difficult to assimilate the nutrient from animal protein and once stomach acid decreased, it will lead to gastritis or stomach inflammation which always accompanies infection (Yahaya *et al.,* 2009).

Furthermore, the relationship between *H. pylori* and sources of drinking water showed that well water has the highest percentage of 48.3%. This result corresponds with the work of Ayuba *et al.,* (2007) on similar study conducted to determine the possible route of the transmission of *H. pylori* infection, who reported prevalence rate of 49%. Nevertheless, these findings disagree with the work of Sani *et al.,* (2013) who reported a low prevalence rate of 19.3%. The reason for the differences could be attributed to the environmental conditions of the study population, factors such as poor hygiene resulting in fecal contamination. According to WHO this could be as a result of wells are mostly dug close to toilets and this gives room for contamination to occur due to proximity of the well to latrine (Fruta, 2010).

The relationship between *H. pylori* infection and Hyperemesis gravidarum observed in the study shows that 72% of the subjects with history of hyperemisis gravidarum were positive to *H. pylori*. This finding is similar to the work conducted in Kebbi, North Western part of Nigeria, by Ahmed *et al.,* (2014), who reported 73.3% of *H. pylori* infection. This result also agrees with the work of Keong and Suleiman, (2016) conducted in Northern part of Nigeria, Kaduna State, who reported that out of 150 pregnant women experiencing severe nausea and vomiting, 74.3% had concurrent *H. pylori* infection (Dogara *et al.,* 2010). The reason for the correlation could be due to the accumulation of fluid and displacement of intracellular and extracellular fluid which may occur as a result of increase in steroid hormones, and this condition results in a change of pH which could lead to the manifestation of a latent *H. pylori* infection in the gastrointestinal tract. The increased level of steroid hormones & human chronic gonadotrophins (HCG) during pregnancy lead to changes in pH and motility of GIT, this change favor activities of *H. pylori* infection (Salahudeen *et al.,* 2011).

The relationship between *H. pylori* and heartburn were observed, the result shows that 58.6% of pregnant women with *H. pylori* did not have heartburn. This result agrees with the work of Adenuga *et al.,* (2011) in a study conducted to determine the relationship between *H. pylori* and dyspepsia patients. A prevalence rate of 57.9% *H. pylori* infection were reported for pregnant women without heartburn. This finding disagrees with the finding of Yinka *et al.,* (2008) who reported lower prevalence of 12.2% of *H. pylori* infection in pregnant women without heartburn. Statistical analysis of the risk factors associated with *H. pylori* infection using chi-square showed no significant association (p-value greater than 0.05) between *H. pylori* infection with pregnancy stages in trimesters, gastrointestinal illness, heartburn, and source of water for drinking. However, this study showed significant association (p-value of 0.03) between *Helicobacter pylori* infection and hyperemesis gravidarum among the study population. This may be due to the sample size used in this study.

CONCLUSION

The result obtained showed a low prevalence of *Helicobacter pylori* infection amongst the pregnant women in the study population, as serology revealed that, out of 100 samples tested and analyzed; only 29% of pregnant women were positive to *H. pylori* infection and 71% were negative. .

RECOMMENDATIONS

1. Further studies are required in large scale in the community using different diagnostic methods to explore the most effective method for detecting the presence of *H. pylori* in pregnant women and to determine whether screening for *H. pylori* infection during pregnancy could benefit the mother and the fetus.
2. There is need to create awareness of *Helicobacter pylori* infection for its possible prevention amongst pregnant women in the study population.
3. Serological kits should be specifically manufactured for local use, the kits should also be available especially in endemic Areas.

Conflict of interest

The authors have not declared any conflict of interests.

REFERENCES

Abubakar, B., Sameer, K. H. and Yesmin, Z. J. (2013). Upper gastrointestinal findings and incidence of *Helicobacter pylori* infection among Nigerian patients with dyspepsia. *West Africa Journal of Medicine*, *20* (2), 140-145

Adenuga, S., Feldman, M., Raheem, B. and Ademolola, L. (2011). Role of seroconversion in confirming cure of *Helicobacter pylori* infection. *Journal of Infectious Diseases, 8* (3), 63-65.

Ahamad, J., Radyan, J. and khaledeen, H. (2014). *). Helicobacter pylori* and the pathogenesis of gastroduodenal inflammation. *Journal of Gastrointestinal Diseases*, *16*(1), 626-633.

Ajose, S. and Naito, H. L. (2009). Adherence of *Helicobacter pylori* to primary Human gastrointestinal cell. *Journal of the Infectious Diseases; Immunology, 6*(3), 451-527.

Alhasan, T., Nuruldeen, M. and Kayode, H. (2009). Hypotheses on the pathogenesis and natural history of *Helicobacter pylori* -induced inflammation. *Gastroenterology, 12* (3), 720-727.

Aslan, S., Abbasalizadeh, S. Darvishi, Z., Abuhaneef, F., Azumar, A., Borji, M. et al.(2013). Prevalence of *Helicobacter pylori* infection among Iranian pregnant women a meta analysis study. *Journal of Knowledge & Health, 11* (7), 23.

Ayuba, D., Atabay, H. and Azaman, B. (2007). *Helicobacter pylori* infection: detection, investigation, and management. *Journals of Pediatrics, 14*(3), 2-6.

Cardaropoli, S., Rolfo, A. and Todros, T. (2014). *Helicobacter pylori* and pregnancy-related disorders. *World Journal of the Gastroenterology, 20* (7), 654-664.

Comfort, D., Austin, Q. L. and Yetunde, S. (2012). The positivity of *Helicobacter pylori* Stool Antigen in patients with Hyperemesis gravidarum. *Journal of Turkish German Gynecology Association, 12* (2), 71-74.

Dogara, G. M., Gobel, R., Symonds, L., Butler, N. and Tran, D. (2010) Disease-specific *Helicobacter pylori* virulence factors: the unfulfilled promise. *Helicobacte*r, *6* (5), 27-31.

Dube, W. H., Cellini, S., Lones, R. and Lesells, F. (2009). Coccoid form of *Helicobacter pylori* in the human stomach. *Journal of Clinical Pathology, 1*(2), 503-507.

Fruta, Y. M. (2014). Role of *Helicobacter pylori* infection in iron deficiency during pregnancy. *American Journal Obsteteric Gynecology*. *3* (6), 420-615.

Imran, A., Abbasalizadeh, F., Azami, D. and Mahmud, N. (2016). Seroprevalence of *Helicobacter pylori* infection in patients suffering from gastric symptoms in the Northwest of Iran. *African Journal of Microbiology Research, 5* (22), 3616-3619.

Kanbay, D. F., Kelly, S. and Thomas, J. E. (2011). Attachment of *Helicobacter pylori* to human Gastric epithelium mediated by blood group antigen. *Journal of Infectious Diseases, 16* (8), 22-26.

Keong, S. F. and Suleiman, G. (2016). Seroprevalence of *Helicobacter pylori* Infection in Patients with Gastritis and Peptic Ulcer Disease in Kaduna, Kaduna State, Nigeria. *African Journal of Basic and Applied Sciences*, 1(*6*), 123-128.

Kitagawa, M., Natori, M., Katoh, M., Sugimoto, K., Akiyama, Y. and Sago, H. (2008). Maternal transmission of *Helicobacter pylori* in the perinatal period. *Journal of Obstetetric Gynecology, 27* (8), 225-230.

Klein, G. F., Lehnthardt, M. and Warren, R. (2012). *Helicobacter pylori*, *Diagnosis and treatment. University of Microbiology* (5th Ed). Western Australia. 231- 236.

Kure, S. K., Hopkins, R. J. and Klein, D. (2012). Seroprevalence of *Helicobacter pylori* Chile: vegetables may serve as one route of transmission. *Journal o f Infectious diseases*, *16*(8), 222–226.

Labignet, G. H., Tailor, D. and Parsonnet, J. (2009). Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *International Agency for Research methodology, 13* (1), 34 – 36.

Mahdavi, J., Sondén, B., Hurtig, M., Olfat, F. O., Forsberg, L. and Roche, N. (2011). *Helicobacter pylori* SabA adhesin in persistent infection and chronic inflammation. *Journal of Sciences,* 2(9), 573 - 578.

Ndububa, Y. O., Correa, P., Forman, R. and Watson, H. E. (2014). Prevalence of *Helicobacter pylori* infection among sewage workers. *Journal of work, Environmental Diagnosis through Serology and Endoscopic Examination, 19*(22), 364 – 368.

Nwodo, J. M., Kikuchi, R., Dore, Y. and Sahay, P. (2012). Axon, ATR. Reservoirs of *Helicobacter pylori* and modes of transmission. *Journal of Helicobacter, 1*(7), 175–182

Poveda, V. N., Nilius, A., Fruta, T. N. and Cellini, S. (2015). Increased susceptibility to *Helicobacter pylori* infection in pregnancy. *Journal of Gastroenterology*, *9* (3),71-83.

Salahudeen, R., Sameer, F. and Tunde, G. (2011). *Helicobacter pylori* infection in South Nigerians: a serological study of dyspeptic patients and healthy individuals. *West Africa Journal of Medicine,* 3 (3), 6-27.

Sani, K., Ernst, A. G. Nazeer, A. and Koren, G. (2009). Relationship between *Helicobacter pylori* infection and gastrointestinal symptoms and syndromes. *Tropical Medicine and International Health, 8*(11), 987-991.

Tanith, Y., Namavar, B. and Dore, R. (2008). Presence of *Helicobacter pylori* in the oral cavity, oesophagus, stomach and faeces of patients with gastritis. *European Journal of* C*linical Microbiology and Infectious Diseases, 14* (2), 27. 37.

Wayne, Q. and Shankar, G. J. (2010). The clinical role of stool test (HpSA) in non invasive diagnosis of *Helicobacter pylori* infection. *Turk Jounal of Gastroenterol, 21*(2), 97-102.

Weyermann, M. D., Brenner, H. and Adler, G. (2016). *Helicobacter pylori* infection and the occurrence and severity of gastrointestinal symptoms during pregnancy. *American Journal of Obstetric Gynaecology, 4*(5), 26-31.

Yahaya, H., Personnet, R. and Sunday, N. (2009). Positive serology for *Helicobacter pylori* and vomiting in the pregnancy. *Archeology, Gynecology and Obsteteric 2*, 10-14.

Yinka, I., Oyindamola, O., Fathi, W. and Adegoke, F. (2008). *Helicobacter pylori* infection in Africa: *Pathology* and microbiological diagnosis in Africa. *Journal of Biotechnology, 19*(22), 364 – 368.