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Seroprevalence of *Helicobacter pylori* in Patients with Diabetes Mellitus in Benin City, Edo State, Nigeria

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ABSTRACT: *Helicobacter pylori* is a Gram-negative microaerophilic bacteria with a spiral (helical) morphology which is usually found in the stomach. *H. pylori* infection is one of the most common bacterial infection worldwide and in children of lower ages. Reports show that this bacterium is associated with simple dyspepsia, heartburn and peptic ulcer diseases, most commonly leading to upper gastrointestinal bleeding and ultimately to the severe compilation of gastric malignancy. Diabetes mellitus is an important factor that increases the susceptibility and risk of various infections in the host. The prevalence of *H. pylori* among diabetes mellitus patients in Benin City and Nigeria has not been studied extensively, thus, this study aimed at determining the seroprevalence of *H. pylori* and assessing the possible risk factors associated with the infection among diabetes mellitus patients in Benin City. Three hundred and fifty (350) serum samples that included two hundred (200) diabetes mellitus samples and one hundred and fifty (150) serum samples from non-diabetics (healthy control) were collected and tested for fasting blood sugar and *H. pylori* antibodies (IgG) using enzymatic technique and immunochromatography test kit respectively. Chi-square test was used to compare diabetes mellitus and control groups (P value less than 0.05 was considered statistically significant). Out of 200 diabetes mellitus patients that were tested for *H. pylori* antibodies, 103 (56.3%) were seropositive. In the non-diabetic control group, out of 150 that were tested 72 (52.6%) were seropositive. P value of 0.500 indicated no statistically significant difference between both groups. In the diabetes mellitus respondent, there was a significant association observed between seropositivity to *H. pylori* infection and fasting blood sugar, sanitary condition, and water supply. In the control group, living in a crowded household was significantly associated with seropositivity to *H. pylori* infection (p=< 0.001). Findings from this study showed that there is no statistically significant association between diabetes mellitus and *H. pylori* infection. Good sanitary conditions as well as properly cooked food and a reliable supply of clean portable water will help to reduce the risk of *H. pylori* infection.

Keywords: *Helicobacter pylori*, Diabetes mellitus, Prevalence, Immunochromatography

Introduction

Helicobacter pylori is a helix-shaped, microaerophilic, flagellated bacterium. With more than 50% of the population being infected, this bacterium is one of the most significant cause of human infection. Humans and *H. pylori* have been in a relationship for at least 50,000 years. *H. pylori* is only known to live in humans. Infection with *H. pylori* is usually acquired in early childhood, and may last a lifetime (Kusters *et al*., 2006). Although more than 80% of infected people have no symptoms (Blaster 2006), the infection may cause gastritis, peptic ulcers, and stomach cancer. The World Health Organization has designated *H. pylori* as the primary cause of stomach cancer, and as a result, classified it as a class one (1) carcinogen in immune-suppressed hosts like Diabetes Mellitus (DM) patients, *H. pylori* is a significant opportunistic bacteria (Khattab *et al*., 2019). The human stomach is thought to be the reservoir for the pathogen and it may spread through the faecal-oral route (Das and Paul, 2007). When the pancreatic beta cells are unable to make insulin or when the body is unable to properly use the insulin that is produced, diabetes mellitus develops. Diabetes lowers the body's defences and raises the possibility of vulnerability to different opportunistic diseases. This particular bacterium inhabit only the stomach, where it causes inflammation and alters the physiology of the stomach. With the help of its binding molecules, which make it easier for the bacterium to adhere to its host, and the astonishing

tools it has developed to directly impact the host cells, *H. pylori* can effectively change its severe environmental niche for its benefit. This bacterium developed ways to actively and passively escape the human immune response through selection and coevolution. Due to its extensive prevalence, its socioeconomic effects, and the global increase in antibiotic resistance rates, novel preventive and treatment strategies will therefore be required.

Materials and methods

Study area: The study was carried out at the University of Benin Teaching Hospital (UBTH) in Benin City, capital of Edo State. It is located at longitude 6.3903° N, and latitude is 5.6118° E and has an estimated population of about 1.125,058 people (Taboola, 2015). It is located in the south-south region of Nigeria. It is bounded in the north by Kogi State, in the west by Ondo State and the south by Delta State. The University of Benin Teaching Hospital (UBTH) is a premier and multi-specialty healthcare service provider in West Africa. The Tertiary Referral Hospital, widely acknowledged as a Centre of Excellence, has remarkably and effectively served as the last port of call for expert management of diverse and varied disease conditions in Edo, Delta, part of Kogi and Ondo State which largely form its catchment area and sometimes further away. The hospital is located in Ugbowo, Benin City and was established on May 12, 1973, following the enactment of an edict (number 12) of the Nigeria National Health Act.
Study population: Participants were drawn from diabetes mellitus patients who attend the diabetic clinic at the University of Benin Teaching Hospital in Benin City, and control subjects were recruited from apparently healthy non-diabetic individuals (with normal blood glucose levels), attending the Outpatient clinic. A total number of 350 participants was recruited for this study which includes 200 diabetic patients and 150 non-diabetic controls. Informed consent was obtained from patients before sample collection.
Research design: This is a case-control study. A well-structured questionnaire was used to collect socio-demographic details such as age, gender, area of residence, occupation, marital status, educational level, Data such as the source of drinking water, sanitary condition, duration of diabetes, fasting blood sugar level, family history of diabetes, glycaemia control, was also collected from the participants.
Specimen collection: Three milliliters (3ml) of venous blood was collected aseptically into plain container tubes. The sample in the plain tube was allowed to clot for 15 minutes and centrifuged at 4000 rpm for 10 min to obtain the serum which was analysed immediately for the presence of *H. pylori* infection using a commercially available *Helicobacter pylori* IgG rapid test kit to detect the presence of *H. pylori* immunoglobulin G (IgG).
Determination of fasting plasma glucose (FPG): Fasting Plasma Glucose (FPG) of each participant was determined using a standardized glucometer following the glucose oxidase peroxidase method (Trinder, 1969). The glucose test strip was inserted into the glucose meter, and 0.6 µl of capillary blood sample was added to the specimen area of the disposable test strip which the meter read and used to calculate the blood glucose level. The meter then displayed the level in units of mg/dl.
Detection of Helicobacter pylori antibodies: Antibodies to *Helicobacter pylori* was detected from sera samples of each participant using an immunochromatographic test kit (One step Anti H. pylori IgG jest Diagnos, China) following the manufacturer's instruction. One hundred microliters (100 ul) of serum was added to the specimen area of the test kit. This was allowed to flow by capillary action and the result was read after 15 min. A red line appearing only in the control area, indicated a negative result. A red line appearing in the control and test area indicated a positive result. This test qualitatively and selectively detects *H. pylori* antibodies in serum or plasma by utilizing a combination of *H. pylori* antigen-coated particles and anti-human IgG.
Statistical analysis: The results were analyzed using the statistical package for the social sciences program (SPSS) version 21. A Chi-square test was used out to compare the Diabetic and Control groups. P-value less than 0.05 ($p<0.05$) was regarded as statistically significant.

Results

The association between *H. Pylori* antibodies and the socio-demographic characteristics of respondents with diabetes mellitus is presented in Table 1. A higher proportion, 46 (62.2), of male DM respondents were seropositive for *H. pylori* compared to the females which is 57 (52.3). This association was not statistically significant ($p= 0.187$). A higher percentage 19 (65.5%) of respondents with seropositivity to *H. pylori* antibody were within the age range of 60-69 years, while the least age range was >70 years. This association was also not statistically significant ($p= 0.106$).

Table 1: Association between *H. pylori* antibodies and socio-demographic characteristics of respondents with diabetes mellitus

Variable	*H. pylori* Antibodies		χ^2	p-value
	Positive (n = 103) Frequency (%)	Negative (n = 80) Frequency (%)		
Sex				
Male	46 (62.2)	28 (37.8)		
Female	57 (52.3)	52 (47.7)	1.745	0.187
Age Group				
40 – 49	21 (63.6)	12 (36.4)		
50 – 59	42 (59.2)	29 (40.8)		
60 – 69	19 (65.5)	10 (34.5)		
>70	21 (42.0)	29 (58.0)	χ^2 = 6.114	0.106
Social Economic Status				
Low	36 (56.3)	28 (43.8)		
Middle	38 (46.9)	43 (53.1)		
High	29 (76.3)	9 (23.7)	χ^2 = 3.088	0.121
Religion				
Christian	102 (57.0)	77 (43.0)		
Muslim	1 (25.0)	3 (75)	Fischer's Exact = 1.627	0.202
Level of Education				
Primary	24 (50.0)	24 (50.0)		
Secondary	22 (44.9)	27 (55.1)		
Tertiary	57 (66.3)	29 (33.7)	χ^2 = 3.848	0.303

The association between *H. pylori* antibodies and the current fasting blood sugar of respondents with diabetes mellitus is presented in Table 2. There was statistically significant association between fasting blood sugar and seropositivity to *H. pylori* antibody (p=0.003). The highest value of blood sugar 15 (75.0%) and 35 (71.4%) of respondents which were seropositive to *H. pylori* were within the range of 127-140 mg/dl and 111-126 mg/dl respectively, and those with the least proportion 0 (0.0%) and 26 (44.1%) were within the range <70 mg/dl and > 140 mg/dl respectively.

Table 2: Association between *H. pylori* antibodies and the current fasting blood sugar of respondents with diabetes mellitus

Variable	*H. pylori* Antibodies		Test statistics	p-value
	Positive (n = 103) Frequency (%)	Negative (n = 80) Frequency (%)		
Current FBS				
< 70mg/dl	0 (0.0)	4 (100)		
70-110mg/dl	27 (52.9)	24 (47.1)		
111-126mg/dl	35 (71.4)	14 (28.6)		
127-140mg/dl	15 (75.0)	5 (25.0)		
> 140mg/dl	26 (44.1)	33 (55.9)	χ^2 = 16.375	0.003

The association between *H. pylori* antibodies and the current random blood sugar of control is presented in Table 3. Majority of the control 29(60.4%) respondents positive to *H. pylori* antibody test had a random blood sugar of <120 mg/dl. The association between the seropositivity of *H. pylori* and random blood sugar of the control respondent was not significant (p= 0.176).

Table 3: Association between *H. pylori* antibodies and current random blood sugar of control

Variable	*H. pylori* Antibodies		Test statistics	p-value
	Positive (n = 72) Frequency (%)	Negative (n = 65) Frequency (%)		
Current RBS				
< 120mg/dl	29 (60.4)	19(39.6)		
120-200mg/dl	43 (48.3)	46 (51.7)	χ^2 = 1.832	0.176

The association between risk factors for acquiring *H. pylori* infection and *H. pylori* seropositivity of Diabetes mellitus respondents is presented in Table 4. Majority of the respondents 64 (85.3%) with poor sanitary

conditions were seropositive to *H. pylori* infection, whereas only 39 (36.1%) with good sanitary conditions were seropositive to *H. pylori* infection. This association was statistically significant (p= <0.001).

Table 4: Association between risk factors for acquiring *H. pylori* infection and *H. pylori* seropositivity of diabetes mellitus respondents

Variable	***H. pylori* Antibodies**		Test statistics	p-value
	Positive (n = 103) Frequency (%)	**Negative (n = 80) Frequency (%)**		
Hygiene level rating				
Middle care	75 (52.8)	67 (47.2)		
High Care	28 (68.3)	13 (31.7)	$\chi^2 = 3.097$	0.078
Sanitary condition				
Good	39 (36.1)	69 (63.9)		
Poor	64 (85.3)	11 (14.7)	$\chi^2 = 43.584$	<0.001
Clean water supply				
Yes	78 (55.7)	62 (44.3)		
No	25 (58.1)	18 (41.9)	$\chi^2 = 0.079$	0.799
Living in crowded household				
Yes	75 (89.3)	9 (10.7)		
No	28 (28.3)	71 (71.7)	$\chi^2 = 68.729$	<0.001

The association between risk factors for acquiring *H. pylori* infection and *H. pylori* seropositivity of the control group is presented in Table 5. Majority of the control group 57 (71.3%) with poor sanitary conditions were seropositive to *H. pylori* infection, while only 15 (26.3%) with good sanitary conditions were seropositive to *H. pylori* infection. This association was statistically significant (p= <0.001). A higher proportion of control respondents 55 (70.5%) living in crowded households were seropositive to *H. pylori* infection, while 17 (28.8%) of the respondent not living in crowded households were seropositive to *H. pylori* infection. This association between living in crowded households and seropositivity to *H. pylori* infection was statistically significant (p= <0.001). There was a statistically significant association (p= <0.001) between water supply and seropositivity to *H. pylori* infection. The highest proportion 33 (80.5%) of control respondents had a poor source of water supply while 39 (40.6%) of them had a clean source of water supply.

Table 5: Association between risk factors for acquiring *H. pylori* infection and *H. pylori* seropositivity of control group

Variable	***H. pylori* Antibodies**		Test statistics	p-value
	Positive (n = 103) Frequency (%)	**Negative (n = 80) Frequency (%)**		
Hygiene level rating				
Middle care	52 (48.1)	56 (51.9)		
High Care	20 (69.0)	9 (31.0)	$\chi^2 = 3.973$	0.046
Sanitary condition				
Good	15 (26.3)	42 (73.7)		
Poor	57 (71.3)	23 (28.7)	$\chi^2 = 26.952$	<0.001
Clean water supply				
Yes	39(40.6)	57 (59.4)		
No	33 (80.5)	8 (19.5)	$\chi^2 = 18.309$	<0.001
Living in crowded conditions				
Yes	55 (70.5)	23 (29.5)		
No	17 (28.8)	42 (71.2)	$\chi^2 = 23.425$	<0.001

The association between *H. pylori* antibodies and duration of diabetes among respondents with diabetes mellitus is presented in Table 6. Majority of the DM respondent 46 (59.7%) with a duration of diabetes >10 years has a greater seropositivity to *H. pylori* antibody, compared to those with a duration of diabetes of 6-10 years 26 (50.0%) and 1-5 years 31 (57.4%) respectively. This relationship was however not statistically significant (P= 0.539).

Table 6: Association between *H. pylori* antibodies and duration of diabetes among respondents with diabetes mellitus

Variable	*H. pylori* Antibodies		Test statistics	p-value
	Positive (n = 103) Frequency (%)	Negative (n = 80) Frequency (%)		
Duration of Having DM				
1 - 5yrs	31 (57.4)	23 (42.6)		
6 - 10yrs	26 (50.0)	26 (50.0)		
> 10yrs	46 (59.7)	31 (40.3)	$\chi^2 = 1.236$	0.539

The relationship between *H. pylori* antibodies seropositivity among respondents with diabetes mellitus (DM) and the control group is presented in Table 7. Out of 183 DM respondents tested for *H. pylori*, 103 (56.3%) were seropositive for *H. pylori* infection while 80 (43.7%) were negative. For the control group, out of 137 tested, 72 (52.6%) were seropositive for *H. pylori* infection while 65 (47.4%) were negative. There was however no statistically significant relationship (P=0.500) between *H. pylori* antibody seropositivity among respondents with Diabetes Mellitus and the Control group.

Table 7: Relationship between *H. pylori* antibodies seropositivity among respondents with diabetes mellitus (DM) and control group

Variable	*H. pylori* antibodies positivity		Test statistics	p-value
	DM (n = 183) frequency (%)	Control (n = 137) frequency (%)		
Seropositivity				
Positive	103 (56.3)	72 (52.6)		
Negative	80 (43.7)	65 (47.4)	T-test	0.500

Discussion

Diabetes mellitus is an important factor that increases the susceptibility and risk of various infections in the host. The prevalence of *Helicobacter pylori* among diabetes mellitus patients in Benin City and Nigeria has not been studied extensively. Thus, this study was conducted to determine the seroprevalence of *Helicobacter pylori* and to assess possible risk factors associated with the infection among diabetic patients in Benin City.

The total prevalence of *H. pylori* among the study participants was 56.3%. This prevalence is higher than the 35% prevalence obtained from a study of *H. pylori* seropositivity in Nigerians with Type 2 diabetes mellitus. (Ugwu *et al*., 2008). The result obtained in this study is lower than 85.6% from a study of the prevalence of *H. pylori* in diabetic patients and health workers at a tertiary hospital in South-west Nigeria (Adeleye *et al., 2019*). This seroprevalence variation may be attributed to differences in methodology, technical factors, variation in age, ethnicity as well as the level of sanitation and social economic status of individual subjects screened.

The prevalence of *H. pylori* antibody in this study was 56.3 % in diabetic patients and 52.6 % in non-diabetic control. The p value of 0.500 indicated no statistically significant difference between both groups. This result is in agreement with the Diabetes Preventive Program (DPP) study obtained by Alzahrani *et al.* (2017) who reported 40% in diabetic patients and 39% in healthy control in the US and of that obtained by Oluyemi *et al*. (2012) in Lagos, Nigeria, who detected *H. pylori* infection in 18% of Type 2 diabetes mellitus (T2DM) patients and 13% of the control, with no statistically significant difference (p= 0.52) recorded). Furthermore, findings on studies on the prevalence of *H. pylori* in diabetes mellitus patients have been contradictory as some studies found that *H. pylori* infection was significantly higher in the DM group compared to the control group (Devrajani *et al*., 2010; Bener *et al*., 2007; Gulcelik *et al*., 2005). Other studies did not find any significant difference in the DM group and control group (Anastasios *et al*., 2002; Ko *et al*., 2001; Stanciu *et al*., 2003).

A higher proportion 46 (62.2%) of DM respondent that was seropositive for *H. pylori* were males compared to 57 (52.3 %) of females. This difference however was not statistically significant (p= 0.187). This finding is consistent with those of de Martel *et al*. (2006) who suggested that the male predominance in *H. pylori* infection might be due to poor hygiene and higher physical activity.

Regarding the impact of age on the seroprevalence of *H. pylori* in diabetes mellitus patients, *H. pylori* seropositivity rate increased gradually with age and peaked age group 60-69 years 19 (65.5 %) after which it showed a decline, the difference in the age group was however not statistically significant (p = 0.106). This result is similar to the findings of Oluyemi *et al*. (2012) who noted that the *H. pylori* positive type 2 diabetes

mellitus patients (T2DM) were slightly-older than *H. pylori*-negative counterparts but the difference was also not statistically significant. (Oluyemi *et al*., 2012).

A higher proportion 15 (75.0 %) of DM respondent that was seropositive for *H. pylori* were having a fasting blood sugar range of 127-140 mg/dl. This relationship was statistically significant (p= 0.003). This is consistent with Upala *et al*., (2016) who systematically reviewed and quantified the effect of *H. pylori* infection on the risk of metabolic syndrome (MS) and metabolic parameters in individuals with *H. pylori* infection, and concluded that *H. pylori* infection is positively associated with higher triglyceride, Body Mass Index and fasting blood sugar (Upala *et al*., 2016).

There was a significant association (p = <0.001) between sanitary conditions and seropositivity of *H. pylori* infection as those with poor sanitary conditions were more positive (85.3 %) compare to those with good sanitary conditions (36.1%). Similarly, in the control group prevalence was higher 51 (71.3 %) with those having poor sanitary conditions compared to those 12 (26.3 %) having good sanitary conditions. This result is consistent with the study of Laporte *et al*. (2004) as *H. pylori* can be spread via fecal contaminants.

Diabetes mellitus respondents living in crowded households showed more seropositivity to *H. pylori* infection (89.3 %) compare to those not living in crowded households (28.3 %) with a statistically significant of p <0.001. Similarly, in the control group prevalence was higher 55 (70.5 %) with those living in crowded households compared to those 17 (28.8 %) not living in a crowded households. This is consistent with a study carried out in Cameroon by Kouitcheu Mabeku and his coworkers, who got a higher rate of 65.32 % in overcrowded households in contrast with a lower rate of 33.33 % from non-crowded households (Kouitcheu Mabeku *et al.,* 2018).

Majority of the DM respondent 46 (59.7 %) with a duration of diabetes > 10 years has a greater seropositivity to *H. pylori* antibody, compared to those with a duration of diabetes of 6-10 years. 26 (50.0 %) and 1-5 years 31 (57.4 %) respectively. This relationship was however not statistically significant. This finding is consistent with the study on Chinese subjects by Ko and his coworkers who found no association between *H. pylori* infection, glycaemic status, and duration of diabetes among diabetic subjects in Hong Kong. (Ko *et al*., 2001).

Conclusion

In this study the difference between *H. pylori* infection rates in Diabetes mellitus patients and the control group was not statistically significant, hence this suggests that *H. pylori* infection is not increased in Diabetes Mellitus.

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