



THE PREVALENCE OF *HELICOBACTER PYLORI* INFECTION AND ITS ASSOCIATION WITH DIABETES AMONG THE PEOPLE OF NORTHERN CROSS RIVER STATE, NIGERIA

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Abstract

Diabetes Miletus is an emerging pandemic disorder with very complex pathogenesis. Studies have blamed it for the annual death of about 3.8 million people worldwide. Among the risk factors for diabetes are diet, physical activity, genetics and obesity, though a number of cases have been found where the recognized risk factors are absent. Recent studies have associated Helicobacter pylori infection with diabetes, though reports are contradictory. This study therefore aimed to determine the prevalence of H. pylori infection in the Northern zone of Cross River State as well as its association with diabetes Miletus. The study employed the cross-sectional survey design. Multistage sampling technique was used to select the subjects. Three Local government areas out of the five in the study area were randomly selected. In the three selected LGAs, three council wards were randomly selected by balloting. Two villages each were randomly selected in each ward; then incidental sampling technique was used to select 20 subjects who met the inclusion criteria. A total of 360 subjects formed the sample size which was determined using Slovin's formula. The blood samples collected were taken to the lab within two hours for analysis (for H. pylori infection and blood sugar levels). Ethical approval for the study was sought and obtained from the Cross River State Ministry of Health. Informed consent was sought from the subjects and anyone who refused was excluded. The data collected were analysed using percentages, chi-square and t-test. Results revealed that H. pylori infection prevalence is 54.7 in the Northern Zone of Cross River State and strongly associates with diabetes ($P < 0.05$). Screening and treatment of diabetes patients for H. pylori as well as adequate measures for its reduction are recommended

Keywords: *H. pylori; diabetes; sugar levels; insulin resistance*

Introduction

H. pylori is a spiral shaped Gram-negative bacterium with 5-7 flagella at one end. It was first isolated in Perth, Western Australia by Barry Marshall and Robin Warren in 1983. To prove that *H. pylori* was related to peptic ulcers, the organism was cultured from the stomach, and after several studies, it was established that *H. pylori* caused peptic ulcers and gastritis (Park, 2011). *H. pylori* infection is very common among humans (about 30 % to 50 % of people in developed countries and about 80 % to 90 % in developing countries) (Campuzano-Maya, 2016). In the U.S., studies have reported the prevalence as low as 7% (Diaz, Regino & Zuleta, 2013). Hussein, (2009) avers that *H. pylori* colonizes all human populations worldwide, with socioeconomic status, geographic area and age playing crucial roles in the risk of colonization and disease development.

The bacterium has been known to be responsible for chronic gastritis, peptic ulcer and increased risk of gastric cancer as well as extra- gastric complications. Even WHO has classified it as a type I carcinogen. It produces chronic gastritis in all cases of infection, though in most patients, the infection is asymptomatic (Eslick, 2019). Diaz *et al.* (2013) aver that there is increasing evidence linking *H. pylori* infection with extra-gastric diseases such as blood, coronary, hepatic and neurological diseases.

Helicobacter pylori infection is a common, affecting about 50% of the world's population. The prevalence is highly variable from country to country (for instance, 75–83% in the Latin American, 39.6% in Japan, and 17.1% in the US (Segal *et al.*, 2008). Abbas *et al.* (2018) set out to determine the prevalence of *H. pylori* infections among schoolchildren in Kasala State in Sudan, using a prospective cross-sectional study design. Their results indicated *H. pylori* infection prevalence of 21.8 %. In 2008, Segal *et al.* investigated the prevalence of *H. pylori* in consecutive children referred to three Canadian tertiary care academic centres for upper gastrointestinal (GI) endoscopy due to upper GI symptoms. Their results indicated a prevalence of 7.1% of which 41.8% were males.

Approximately 3.8 million people die of diabetes type 2 annually worldwide (Nordqvist, 2012). It is an emerging pandemic with a complex pathogenesis. The risk factor include lifestyle, (such as diet, obesity, physical activity etc.), genetic background and socio-economic factors (Yang and Lu, 2014). There have been contradictory reports on association between *H. pylori* and type 2 diabetes. Mabeku *et al.* (2020) evaluated the presence of *H. pylori* infection among dyspeptic diabetic patients in the littoral region of Cameroon, using a cross-sectional study design. The study consisted of 93 type 2 dyspeptic diabetic patients and 112 non-diabetic dyspeptic patients, who were screened for type two diabetes and *H. pylori* infection. Their results showed that 73.11% of diabetic patients were infected with *H. pylori* against 58.05 of non-diabetic patients which difference was significant (P=0.0279). Diabetes

is an emerging pandemic with a complex pathogenesis (He *et al.* 2014). Mansori *et al.* (2020) conducted a systematic review on the association of *H. pylori* with diabetes. They analysed data from 41 studies with a total of 9559 subjects and they concluded that there exists a positive association between *H. pylori* and diabetes mellitus.

Vafaeimanesh *et al.* (2014) evaluated the association of *H. pylori* with insulin resistance and type 2 diabetes using a cross-sectional study. They obtained samples from 211 diabetic patients who were referred to a diabetic clinic (Shahid Beheshti Hospital of Qom) and 218 non-diabetic patients. *H. pylori* infection was determined using serology while insulin resistance was determined using HOMA-IR. Among diabetic patients, prevalence of *H. pylori* infection was 55.8% as against 44.2% for non-diabetic patients, which was statistically significant (P = 0.001).

According to Yang and Lu (2014), emerging data seem to indicate a strong relationship between *H. pylori* infection and the incidence of type 2 diabetes mellitus. Nodoushan and Nabavi (2019) aver that among patients referred to diabetes clinics, about 75% of them usually complain of gastrointestinal symptoms (such as dyspepsia, abdominal pain, nausea, vomiting, and diarrhea), and in many cases, the patients are not properly diagnosed and are undertreated because the GI tract has not been conventionally linked with diabetes. He *et al.* (2014) assert that while the connection between *H. pylori* infection and diabetes is yet to be fully understood, the blame is mostly on inflammatory substances initiated by the infection. According to them, inflammatory substances (such as C-reactive proteins-CRP, IL-6, IL-1 β , and TNF- α) initiated by *H. pylori* infection cause insulin resistance which leads to diabetes. The gastritis caused by the infection also interferes with gastric hormones leading also to insulin resistance and hence diabetes (He *et al.*, 2014).

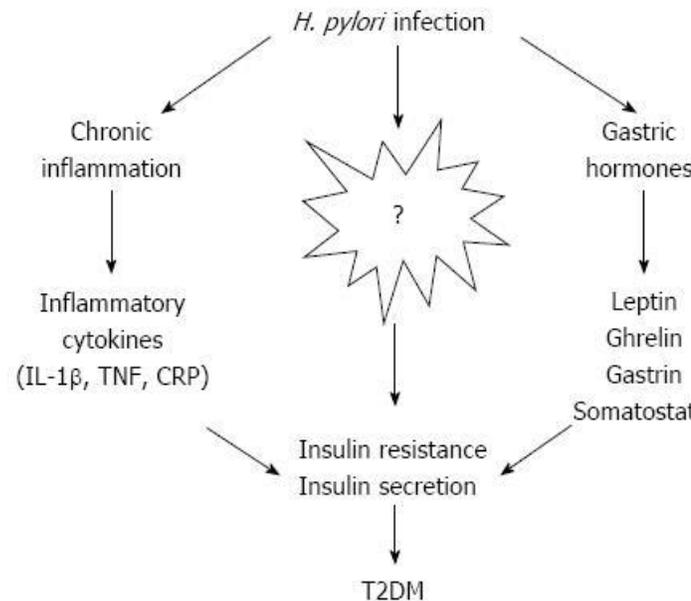


Fig. 1: Potential mechanisms for contribution of *Helicobacter pylori* to type 2 diabetes mellitus (He et al, 2014)

Statement of the Problem:

Infection with *Helicobacter pylori* has been recognized as a public health problem worldwide (Kareem, 2013). Studies have shown that the prevalence rate of *H. pylori* is high worldwide, though much higher in developing countries than the developed world (Olokoba et al., 2013). Studies have associated *H. pylori* infection with gastritis, peptic ulcer, gastric cancer and extra-gastric complications including dyslipidemia, diabetes and heart diseases (Park, 2011). The prevalence also varies among geographical areas, age and socio-economic status. The delayed detection and treatment of *H. pylori* can lead to serious consequences and complications. It is therefore important to find out the regional *H. pylori* prevalence in Cross River North and identify high risk populations in order that treatment strategies can be planned and implemented to reduce the menace of this disease. Reports of investigation on association of *H. pylori* with most of these disorders are still contradictory. What could be the prevalence in the Northern zone of Cross River State? How does the prevalence vary with age and socio-economic status? Is infection with *H. pylori* also associated with blood sugar levels? These questions and more, coupled with the paucity of data on *H. pylori* in the study area are the motivators for this study.

The aim of this study was to find out the prevalence of *H. pylori* infection among the people of Northern of Cross River State, as well as its association with blood cholesterol and sugar levels.

Specifically, the study sought to:

- I. Investigate the prevalence of *H. pylori* in the Northern zone of Cross River State
- II. Find out if *H. pylori* infection is associated with blood sugar levels.

Materials and Methods:

Study Area:

This study was carried out in the Northern senatorial zone of Cross River State. The area is located in the north extreme of the state, and consists of five (5) Local Government Areas which are: Obanliku, Obudu, Bekwara, Ogoja and Yala. The area is bounded with the Republic of Cameroon in the East, Benue state in the North, and Ebonyi state in the West. In the south, the area is bounded by Boki and Ikom of the central zone of the state. According to National Bureau of Statistics (2007), the population of the people in the area as estimated by the National population commission in 2006 is seven hundred and fifty-nine thousand, eight hundred and sixty-two (759, 862) people (Bekwara-105,641; Obanliku-109,633; Obudu-161,457; Ogoja-171,574 and Yala-211,557).

Study Design:

The study employed the cross-sectional observation analytical research design whereby a cross-section of the people was selected based on the sampling technique used, since the researcher did not manipulate any variable.

Study Population:

The population of the study included all the people of the Northern Cross River state (including males and females of all socio-economic backgrounds aged thirteen and above).

Selection of Sample and Sample Size:

The Sample size was determined using the Slovin's formula, as recommended by Ellen, (2020) to be 400 subjects.

Inclusion Criteria: The criteria for inclusion in the study were:

- i. Every person who consented to participate in the study.
- ii. People who were thirteen years and above

Exclusion Criteria:

- i. Individuals known to have been taking antibiotics and protein pump inhibitors in the last two weeks were excluded.
- ii. Individuals who refused to participate were excluded
- iii. People who were less than thirteen years

Informed Consent:

An informed consent form was developed which briefly explains the purpose of the study and its importance (see appendix I). This was given to the prospective participants to show their willingness to participate in the study by signing the form. Those who refused to sign were not included in the study.

Ethical Approval:

The ethical approval for the study was sought for and obtained from the Cross River State Ministry of Health (see attached appendix III)

Sample Collection:

Blood samples were collected for analysis from the vein using sterile needles and syringes. All samples were transported to the laboratory unit of the Catholic Maternity hospital (CMH), Ogoja for storage and subsequent analysis.

Materials:

The materials used in the study included:
Abnova *H. pylori* ELISA kit

CellBiolabs' STA-680 Glucose Assay kit.

Tests:

The following tests were conducted as follows:

1. Whole blood serology test for *H. pylori* antibodies in serum:

This was performed using the Abnova *H. pylori* IgG ELISA kit

According to Abnova (2021), this kit is for evaluating the serologic status of *H. pylori* in patients with gastrointestinal symptoms.

Procedure:

- a. The Serum sample was prepared from a whole blood specimen obtained by acceptable medical techniques.
- b. The desired number of coated wells were secured in the holder.
- c. 1:40 dilution of test samples, negative control, positive control, and calibrator were prepared by adding 5 µl of the sample to 200 µl of sample diluent and mixed well.
- d. 100 µl of diluted sera, calibrator, and controls were dispensed into the appropriate wells and the holder tapped very well to remove air bubbles.
- e. The set up was incubated at room temperature for 30 minutes.
- f. Liquid was removed from all wells and washed with diluted wash buffer and distilled water
- g. 100 µl of enzyme conjugate was dispensed into each well and mixed gently for 10 seconds.
- h. The set up was incubated again at room temperature for 30 minutes.
- i. The unbound enzyme conjugate was removed from all wells by washing off using diluted wash buffer and then with distilled water.
- j. 100 µl of TMB Reagent was then added to each well and mixed gently for 10 seconds.
- k. The set was incubate again at room temperature for 20 minutes.
- l. 100 µl of Stop Solution was then added to each well and mixed gently for 30 seconds, ensuring that all the blue color changes to yellow color completely.
- m. The optical density was read at 450 nm with a microtiter plate reader.

N/B: Care was taken during the wash procedure because insufficient washing results in improper color development (Abnova, 2021).

Results: *H. pylori* IgG EIA Index of less than 0.90 was taken as negative. *H. pylori* IgG EIA Index of 1.00 or greater was taken as seropositive. : *H. pylori* IgG EIA Index between 0.91-0.99 was equivocal

2. **Blood sugar level:** This was performed using the CellBiolabs' STA-680 Glucose Assay kit. **Procedure:** The procedure of the assay was as follows, according to the manufacturer.
 - a. All reagents were prepared and mixed thoroughly before use. Each sample (including the standard and the unknown) was assayed in duplicates
 - iii. 50 µl of each glucose standard and sample were added into wells of 96-well microtiter plate
 - iv. 50 µl of reaction mixture were added to each well and the well contents mixed thoroughly and then incubated at 37°C for 30-45 minutes and protected from light
 - v. The plate were then read using a spectrophotometric microplate reader in 540-570nm range
 - vi. The concentration of glucose in the samples was calculated by comparing the sample OD to the standard curve.

Results from these tests were entered in the *H. pylori* infection and blood glucose level data schedule (HpBGL) (see appendix I).

Data Management:

The data collected were coded and entered in SPSS and analyzed according to the stated research questions using chi square, and t-tests.

Ethical Approval:

The ethical approval for this study was sought for and obtained from the Ethical committee of the Cross River State ministry of Education, Calabar (see attached appendix II).

Results and Discussion:

Prevalence of *H. pylori* in the Northern zone of Cross River State:

The results of data analysis reveal the *H. pylori* infection rate as 54.7% (211 out of the 386 subjects) against 175 (45.3%) that were negative (see table 1 below. This prevalence rate is close to that obtained by Mbang *et al.* (2019) who reported 42.6% prevalence from their study in Calabar. The result is also in line with the findings of Kumurya (2015) who reported a prevalenc of 53% in Kano, Nigeria. The findings also agree with the assertions of Campuzano-Maya (2016); Hussein (2009); and Diaz *et al* (2013) that prevalence of *H. pylori* is

high worldwide but usually higher in the developing countries. The high prevalence may be attributed to low socio-economic status, poor hygiene and sanitation, poor water supply etc. as averred by Brown (2000), Jamkhande *et al* (2016), and Aitila *et al*. (2019).

Table 1: Prevalence of *H. pylori* in the Northern Zone of Cross River State:

INFECTION STATUS	FREQUENCY	PERCENT	VALID PERCENT
POSITIVE	211	54.7	54.7
NEGATIVE	175	45.3	45.3
Total	386	100.0	100.0

***H. pylori* infection and blood glucose levels:**

This was determined by comparing blood sugar levels between *H. pylori* negative and positive subjects using independent t-test. The result is presented in Table 2 below.

Table 2: Independent t-test of blood glucose levels by *H. pylori* infection

<i>H. pylori</i> infection	N	Mean	STD	T	P-value
Positive	211	125.77	42.79		
Negative	175	77.58	20.68	13.63*	0.000
Total	386	103.92	42.06		

*Significant at 0.05

The mean blood sugar level for subjects that were *H. pylori* positive was 125.77, against 77.58 for those who were negative. This difference was tested for significance using independent t-test and the result was significant at 0.05 alpha level. The results of the analysis showed that subjects who were positive for *H. pylori* had a significantly higher blood sugar level than the negative subjects. This shows that infection with *H. pylori* is significantly associated with high blood sugar levels ($P < 0.05$). Going by Mayo clinic's assertion (2023), the mean blood sugar level of *H. pylori* positive subjects (125.77mg/dl) was in the range of diabetes (>125.00 mg/dl). The mean blood sugar levels of those who were *H. pylori* negative was in the normal range (<100 mg/dl).

This result is in line with the results of Mabeku *et al* (2020) and Vafaeimanesh *et al* (2014). Mabeku *et al* (2020) found out from their cross-sectional study in Cameroon that there was a significant difference in the prevalence of *H. pylori* infection between diabetic and non-diabetic patients. Vafaeimanesh *et al* (2014) also found out that the rate of infection with *H. pylori* was significantly higher in diabetic patients than in non-diabetic patients. This result also aligns with the report of Mansori *et al* (2020) from their systematic review where they concluded that there exists a strong positive association between *H. pylori* and diabetes mellitus.

According to Yang and Lu (2014), emerging data seem to indicate a strong relationship between *H. pylori* infection and incidence of type 2 diabetes mellitus. Although the involvement of the bacterium in the pathogenesis of diabetes mellitus type 2

is yet to be fully understood, Yang and Lu (2014) assert that it is largely by inflammation. Usually, the inflammation is by elevations in inflammatory cytokines levels such as C-reactive protein (CRP), interleukin-6, TNF- α etc. (Mansori *et al* (2020). These inflammatory molecules interfere with the ability of cells to respond to instructions from insulin, leading to insulin resistance, resulting in elevated blood sugar levels. The gastritis caused by *H. pylori* infection usually affects the gut-related hormones (leptin, ghrelin, gastrin and somatostatin) which also affect insulin sensitivity and glucose homeostasis (He *et al*, 2014). These have been implicated in insulin resistance and diabetes type 2.

Conclusion and Recommendations:

From the results of this study, it can be concluded that there is a high prevalence of *H. pylori* infection among the people of the Northern zone of Cross River State, and the prevalence is strongly associated with high blood sugar levels. It is recommended that cases of diabetes type two should also be screened and treated for *H. pylori* infection as a possible way of managing diabetes.

Acknowledgements:

The researchers greatly appreciate Mr. Joseph Adie who assisted in the collection of samples as well as Mr. Onyeledo of the Laboratory unit, Catholic Maternity hospital (CMH), Ogoja who helped in the analysis of the blood samples for *H. pylori* and blood sugar levels.

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Article inflow

Received: 5th October, 2025

Accepted: 30th November, 2025

Published 31st December, 2025

