Prevalence of *Helicobacter Pylori* Infection among HIV-1 Infected Patients using Stool Antigen Tests in Jos, North-Central, Nigeria


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**Background:**
*Helicobacter pylori* (*H. pylori*) infection is common among humans and plays a major role in the etiology of peptic ulcer disease with significant morbidity in patients with HIV-1 on antiretroviral therapy. There are conflicting prevalence patterns of *H. pylori* in HIV-1 infected patients using various methods of detection. The noninvasive technique used for detection of *H. pylori* infection is inexpensive and convenient with no complications.

**Materials and Methods:**
We aimed to determine the prevalence of *H. pylori* infection among patients infected with HIV-1 on antiretroviral therapy using *H. pylori* stool antigen. 139 patients infected with HIV-1 were recruited, stool samples were collected and the *H. pylori* stool antigen (HpSA) test was used to detect *H. pylori* antigen.

**Results:**
46.8% of the respondents were positive for *H. pylori* and 53.2% were negative, 18 (13%) were men and 47 (33.8%) were women. HpSA is a relatively simple, inexpensive, and time-saving non-invasive test for the detection of *H. pylori* infections in patients infected with HIV-1.

**Conclusion:**
We also observed that the prevalence of *H. pylori* was low in these patients compared with the general population. However, more studies using *H. pylori* stool antigen test are needed in these patients in the North-Central, Nigeria to further evaluate the infection rate.

**Keywords:** Prevalence, *Helicobacter pylori*, Stool antigen, Enzyme immunoassay, HIV-1 infected

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**INTRODUCTION**

*Helicobacter pylori* (*H. pylori*) infection is amongst the most common infections in humans strongly associated with peptic ulcer diseases, gastric cancer, and lymphomas(1). Approximately two-thirds of the world’s population is infected with *H. pylori*; being more prevalent among older people in the United State of America, African Americans, Hispanics, and lower socio-economic groups(2,3). The pathogen was
first isolated and cultured from the antrum of patients with gastritis, and is a spiral-shaped bacterium commonly found in the gastric mucous layer or adherent to the epithelial lining of the stomach. The prevalence of \textit{H. pylori} infection is more than 50% in developing countries, although the infection rates in most developed nations are dropping. The percent of \textit{H. pylori}-associated gastric cancer is about 6.2% of all cancers, including 89% of non-cardia gastric cancer cases worldwide. However, the rates could be underestimated due to misdiagnosis or low sensitivity of detection of anti-\textit{H. pylori} antibodies(4,5). The gastrointestinal tract (GIT) is the largest immunological site of the body where Human Immunodeficiency Virus type 1 (HIV-1) is known to multiply greatly, and also serves as sanctuary site with attendant gut malfunctions(6). The common ulcer symptom is heart burning or gnawing in the epigastrium. The associated pain occurs typically when the stomach is left empty of food or drink, it can be between meals and in the early hours of the day. This pain may last from minutes to hours, may be relieved by eating or by taking antacids. The relationship between the bacteria and clinical manifestations is well known and it is associated with the presence of the cagA gene that triggers the pathogenicity island; such as Bab A, OIpA, and the carriers of the vacA gene. Studies have reported that only the cagA and vacA gene carriers are most likely to cause ulcer disease(7, 8). Smith et al has reported the relationship of vacA s1, iceA1 and cagA as common genotypes of \textit{H. pylori} infection and duodenal ulcers in Nigeria(9). Other studies have reported the prevalence of \textit{H. pylori} in general population, children and also antibiotic susceptibility patterns in Nigeria(10), and developed countries(11,12).

It is well-known that \textit{H. pylori} infection is mostly acquired during the early years of life and persists for several years in both developed and developing countries(13). About 25 million Americans suffer from peptic ulcer disease at some point in their lifetime, with little documented data in sub-Saharan Africa. More than 650,000 to 850,000 new cases of peptic ulcer disease and more than 1.5 million ulcer-related hospitalizations each year have been reported(14,15).

There are two broadly categorized available methods to detect \textit{H. pylori} infection: invasive and noninvasive methods. The invasive tests include: histology and culture. The cost and discomfort associated to the patients are very high and biopsy samples may be subject to errors related to sampling and interference of contaminated bacteria. The noninvasive tests include: urea breath test (UBT) and serology(16). The use of serological tests in most diagnostic laboratories and hospitals have some drawbacks; they do not discriminate between current and past infections, have low specificity and paucity of data on the specificity and sensitivity in HIV-infected individuals with immunodeficiency with perhaps altered production of antibody(17). The long-term retention of the antigen may cause false positive after the eradication of \textit{H. pylori} because the \textit{Helicobacter pylori} Stool Antigen (HPSA) tests does not distinguish between live and dead bacteria, however, comparative diagnostic methods of \textit{H. pylori} have also reported the usefulness of HPSA in epidemiological findings in Nigeria(18).

The use of stool antigen as a noninvasive rapid test practical tool for detection of \textit{H. pylori} infection is even more desirable in children. Stool antigen tests have recently been welcomed with great expectations as they are convenient and require little technical expertise to perform even in less equipped laboratories(19). However, the accuracy of stool antigen tests in different clinical set up outside of controlled studies posed a challenge, but HpSA GeneFront’s ELISA VUETM (GeneFrontInc, USA) happens to be one of the most widely studied and it has shown to have acceptable performance in the detection of \textit{H. pylori} infection(20). The stool polyclonal and monoclonal antigen tests have high sensitivity, specificity and accuracy in children and HIV-1 infected patients(21).

HIV-1 infected patients experience many forms of opportunistic infections including gastrointestinal symptoms(22). The exact role of \textit{H. pylori} infection among HIV-1infected patients in gastro duodenal lesions might be different from the general population, and it remains unclear if upper gastrointestinal symptoms such as dyspepsia are highly active antiretroviral therapy (HAART) related adverse effects or as a result of \textit{H. pylori} infection. The involvement of gastrointestinal tract may be because it represents the largest reservoir of HIV in the body with attendant...
morphological changes in the upper-gastrointestinal tract mucosa and medication complications (23).

Studies have shown that the prevalence of H. pylori infection in HIV-1 positive patients is remarkably low when compared with the general population (24). Review studies have shown low prevalence of H. pylori infection in HIV-1 infected patients therefore, the use of the stool antigen test is recommended for targeted high risk populations (25). We aimed to determine the prevalence of H. pylori using stool antigen by enzyme immunoassay among HIV-infected adults receiving highly active antiretroviral therapy (HAART) in Jos University Teaching Hospital (JUTH), Nigeria.

MATERIALS AND METHODS

Study Area and Population

The study was carried out at the HIV/AIDS treatment Centre of JUTH in collaboration with the AIDS Prevention Initiative in Nigeria (APIN) program. This clinic provides comprehensive HIV care services for the city of Jos and its metropolis. The clinic also serves as a referral center for health facilities in other Local Government Areas (LGAs) of the state, north central and neighboring states. The subjects used for this study include 139 HIV infected men and women aged ≥17 years receiving highly active antiretroviral therapy (HAART) at the centre.

Study design and study population

This was a cross-sectional study, and 139 HIV-1 infected volunteers accessing care at the JUTH antiretroviral clinic were enrolled. All patients included in the study provided written informed consent for the use of their data for research as approved by the institutional review board of Jos University Teaching Hospital (Health Research Ethic Committee) and were aged ≥17 years.

Data collection

Data collection lasted for four weeks. Consecutive eligible respondents seen over the study period were solicited to participate. Structured questionnaires was used to obtain information on socio-demographic characteristics of the respondents which included: age, sex, marital status, pet keeping, source of drinking water, sanitary practices, alcohol consumption, smoking habit, occupation, housing accommodation, and signs of dyspepsia. Stool samples were collected from the patients with the aid of sterile stool containers with a spatula in them. The sterile spatula was attached to the cover and it was used to take the stool sample from tissue paper to avoid contamination from the toilet and it was covered immediately to avoid drying. Containers were labeled with the patients’ identity number corresponding with the patient’s identity on the questionnaire. Some samples were collected on same day while others were collected on the following day for convenient reasons. All samples were gathered and stored together at -80°C until enough samples were collected which lasted for a period of four weeks.

Laboratory Procedure: Helicobacter pylori Stool Antigen (HpSA ) Test

An approximate peanut size of fresh stool sample were collected and stored at -20°C for analysis. The H. pylori polyclonal stool antigen-based GeneFront’s ELISA VUETM (GeneFrontInc, Kukatpally, Hyderabad 500072, India) was performed according to manufacturer’s instructions. The test is quantitative, and based on a sandwiched enzyme immunoassay for antigen detection with purified H. pylori antibody coated on the surface of micro-wells. One hundred and ninety two coated strips were placed into the holder and 10μl of treated sample calibrators and controls were dispensed into the appropriate wells. Air bubble was removed by tapping the holder from the liquid and it was mixed well. The stripe and the holder were incubated at room temperature for 30 mins. First, 100 μl of a diluted stool sample (10 μl stool in 0.5 ml sample diluent) and thereafter, peroxidase-conjugated polyclonal antibody solution were added to the wells and incubated for 30 mins at room temperature. The unbound material was removed by washing, and washing was repeated 3 times with washing buffer. 100μl of enzyme conjugate was dispensed into each well and incubated at room temperature for 30 mins. After addition of a TMB chromogenic substrate, 100 ml solution was dispensed into each well and was incubated for 15 mins at room temperature and 100μl of stop solution was added to stop reaction, and the intensity of the color generated is proportional to the amount of antigen in the sample. The optical density was read at 450nm by a spectrophotometer compared in a parallel manner with calibrator and controls. The
cutoff OD value for sero-positivity was >20ng/ml and negative <15ng/ml.

**Statistical Analysis**

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 16.0. Standard descriptive statistics were used to examine the sero-prevalence, while associations between socio-demographic characteristics, health status, and variables of potential exposure (environmental and lifestyle conditions), and *H. pylori* sero-positivity of HIV-1 infected patients were examined using bivariate analysis and Chi-square. \( P \leq 0.05 \) was considered as statistically significant.

**RESULT**

Of 139 diagnosed HIV-1 infected patients who participated in the study, the prevalence rate of *H. pylori* infection was 46.8%, 18 (13.0%) were men and 38 (33.8%) were women.

With respect to age category, 17-26 year-olds had the highest frequency (15.1%, n=21), followed by 27-36 year-olds (12.2%, n=17), 37-46 year-olds (11.0%, n=15), and ≥41 year-olds (8.6%, n=12). 38 (27.3%) were married predominated and 27 (19.4%) were single. Most (25.2%, n=35) were unemployed, 21 (15.1%) worked in the private sector or were traders, and 9 (6.5%) were public servants. 27 (19.4%) patients had a history of alcoholic consumption and 7 (5.0%) smoke cigarettes. 29 (21.0%), 11 (8.0%), and 25 (18.0%) of the patients used the pit latrine, water system, and bush for sanitary purposes, respectively (\( P=0.05 \)). 45 (32.4%) had room accommodation and 20 (14.4%) had flat apartments. 14 (10.1%), 12 (8.6%), and 39 (28.0%) patients used tap water, borehole, and well for drinking water, respectively.

Amongst those with signs of dyspepsia, abdominal pain was seen in 35 (25.2%) patients, followed by belching in 11 (8.0%), vomiting 2 (1.4%), heart burn 10 (7.2%), and bloating in 2 (1.4%) patients. 5 (3.6%) patients had no symptoms. 28.8% of the patients kept a pet (\( p=0.30 \)). Although this association was not statistically significant, but the odds of having *H. pylori* infection was about one and half times more in those keeping pets compared with those not keeping pets (OR=95%, CI=1.4, 0.73-2.82). There were no significant differences between *H. pylori* infection in the patients’ with respect to age, marital status, occupation, alcohol consumption, smoking habit, housing accommodation, and drinking water source, (table 1).

**DISCUSSION**

The International Agency for Research on Cancer (IARC) reported that *H. pylori* is a type I carcinogen, or a cancer definite in humans. This report was based almost exclusively on epidemiological evidence, though controversial. It has been argued in some quarters that *H. pylori* is only a risk factor(26). Gastric cancers are a leading cause of cancer morbidity and mortality worldwide; with adenocarcinomas arising from gastric glands accounting for 90% of incident cases(27). The stomach wall is being protected from the gastric juice by thick mucus layer of the stomach lining. *H. pylori* take advantage of this layer by living in the mucus lining. Earlier reported findings showed that more than half of the world's population is infected with *H. pylori*, which is acquired during the early years of life. Actual infection rates vary from nation to nation; the developing countries have much higher infection rates (90%) than the developed countries (1.2-12%)(28). *H. pylori* infection has been known to be associated with gastritis, duodenal ulcer, gastric cancer, and mucosa associated lymphoid tissue lymphoma(17,34). *H. pylori* infection may be asymptomatic; therefore, we included in our study patients with and without digestive complaints, since it may provide better chances for the pathogen detection. Our results showed low prevalence of *H. pylori* infection (46.8%) in HIV-1 patients which differs remarkably from that previously reported study for HIV-negative adults in Nigeria (70.3%) (18) and Brazil (82.0%) (24). This study was stool antigen based, but we observed low prevalence against the earlier reported study in Nigeria 36.7%(29). Similarly, studies have reported different prevalent rates using sera (*H.pylori* IgG antibodies) in Ibadan (82.7-94.5%), Abeokuta (47.8%), Ile-Ife (73.0 %) and Lagos (93.6%), all in South western Nigeria(30-32). Holcombe et al who used histology, Haematoxilllin and Eosin, with modified Giemsa staining of antral biopsies in Maiduguri found a prevalence rate (84%) of *H. pylori* among dyspeptic patients(33).

Also earlier reported findings showed that the
Table 1: Socio-demographic characteristics of Helicobacter pylori infection among HIV-1 Patients attending Jos University Teaching Hospital, Nigeria (n=139).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Number positive (%)</th>
<th>Number negative (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age Category</strong></td>
<td></td>
<td></td>
<td>0.570</td>
</tr>
<tr>
<td>17-26</td>
<td>21(15.1)</td>
<td>18(13.0)</td>
<td></td>
</tr>
<tr>
<td>27-36</td>
<td>17(12.2)</td>
<td>14(10.1)</td>
<td></td>
</tr>
<tr>
<td>37-46</td>
<td>15(11.0)</td>
<td>13(9.3)</td>
<td></td>
</tr>
<tr>
<td>≥41</td>
<td>12(8.6)</td>
<td>29(21.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td>0.600</td>
</tr>
<tr>
<td>Male</td>
<td>18(13.0)</td>
<td>21(15.1)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>47(33.8)</td>
<td>53(38.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
<td>0.060</td>
</tr>
<tr>
<td>Single</td>
<td>27(19.4)</td>
<td>35(25.2)</td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>38(27.3)</td>
<td>39(28.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Occupation</strong></td>
<td></td>
<td></td>
<td>0.600</td>
</tr>
<tr>
<td>Public servant</td>
<td>9(6.5)</td>
<td>38(27.3)</td>
<td></td>
</tr>
<tr>
<td>Private/Trading</td>
<td>21(15.1)</td>
<td>27(19.4)</td>
<td></td>
</tr>
<tr>
<td>Unemployed</td>
<td>35(25.2)</td>
<td>9(6.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Alcohol consumption</strong></td>
<td></td>
<td></td>
<td>0.100</td>
</tr>
<tr>
<td>Yes</td>
<td>27(19.4)</td>
<td>30(21.6)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>38(27.3)</td>
<td>44(31.6)</td>
<td></td>
</tr>
<tr>
<td><strong>Smoking habit</strong></td>
<td></td>
<td></td>
<td>0.200</td>
</tr>
<tr>
<td>Never</td>
<td>58(42.0)</td>
<td>66(47.5)</td>
<td></td>
</tr>
<tr>
<td>Currently</td>
<td>7(5.0)</td>
<td>7(5.0)</td>
<td></td>
</tr>
<tr>
<td>Past</td>
<td>0(0.0)</td>
<td>1(0.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Sanitary practices</strong></td>
<td></td>
<td></td>
<td>0.050</td>
</tr>
<tr>
<td>Pit</td>
<td>29(21.0)</td>
<td>26(18.7)</td>
<td></td>
</tr>
<tr>
<td>Water system</td>
<td>11(8.0)</td>
<td>40(28.8)</td>
<td></td>
</tr>
<tr>
<td>Bush</td>
<td>25(18.0)</td>
<td>8(5.7)</td>
<td></td>
</tr>
<tr>
<td><strong>Housing accommodation</strong></td>
<td></td>
<td></td>
<td>0.090</td>
</tr>
<tr>
<td>Room</td>
<td>45(32.4)</td>
<td>40(28.8)</td>
<td></td>
</tr>
<tr>
<td>Flat</td>
<td>20(14.4)</td>
<td>34(24.5)</td>
<td></td>
</tr>
<tr>
<td><strong>Source of drinking water</strong></td>
<td></td>
<td></td>
<td>0.060</td>
</tr>
<tr>
<td>Tap</td>
<td>14(10.1)</td>
<td>34(24.5)</td>
<td></td>
</tr>
<tr>
<td>Borehole</td>
<td>12(8.6)</td>
<td>19(13.7)</td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>39(28.0)</td>
<td>21(15.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Signs of dyspepsia</strong></td>
<td></td>
<td></td>
<td>0.070</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>35(25.2)</td>
<td>38(27.3)</td>
<td></td>
</tr>
<tr>
<td>Belching</td>
<td>11(8.0)</td>
<td>15(11.0)</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td>2(1.4)</td>
<td>8(5.7)</td>
<td></td>
</tr>
<tr>
<td>Heartburn</td>
<td>10(7.2)</td>
<td>4(3.0)</td>
<td></td>
</tr>
<tr>
<td>Bloating</td>
<td>2(1.4)</td>
<td>7(5.0)</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>5(3.6)</td>
<td>2(1.4)</td>
<td></td>
</tr>
<tr>
<td><strong>Pet keeping</strong></td>
<td></td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>Yes</td>
<td>40(28.8)</td>
<td>39(28.0)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>25(18.0)</td>
<td>35(25.2)</td>
<td></td>
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</table>
prevalence of *H. pylori* infection has been decreasing over the years in the general population(34) but this assertion cannot be easily verified because of the variability of research methodologies employed. However, studies from Kenya, Cameroon and Nigeria also reported higher prevalence of 60% to 71% in dyspepsia patients and in apparently healthy individuals. Similar higher prevalence was reported in Western Nigerian in patients with duodenal ulcers (>90%)(35-38).

Our study is consistent with similar reported findings in HIV-1 infected patients from Western Nigeria (47.4%)(39), though lower prevalence (36.7%) of *H. pylori* infection using HpSA had also been reported in Nigeria among HIV-1 negative individuals(29). Studies from other parts of the world have reported lower prevalence in HIV infected individuals (11.36%, and 22.1%)(40,41).

This study revealed that high proportion (15.1%) of participants aged 17-26 years were infected with *H. pylori* as compared to older age categories, and this was consistent with earlier reported findings in Nigeria(36), and Turkey(42). Similarly, the study observed high proportions of *H. pylori* infection among the women with no significant risk of association, though larger percentage of women had participated compared to men. Poor sanitary practices and signs of dyspepsia among the participants were found to have a significant association with *H. pylori*. The high prevalence of *H. pylori* infection among those that used pit latrine and bush could be attributed to poor socio-economic status. However, studies have documented a relation between low socio-economic status and the high rates of *H. pylori* infection in other parts of the world and Nigeria(34,37). The significant association of signs of dyspepsia among HIV-1 infected patients we observed in our study was consistent with earlier reported studies in Nigerian adults on suppressive antiretroviral therapy(39). The role of *H. pylori* in gastrointestinal disturbances and dyspepsia related symptoms has been documented which is as a result of immunosuppression and therapy indications(6,41).

In our study, the lack of a significant association may be due to a small sample size. This was similar to earlier findings that acquisition of *H. pylori* infection is common through poor environmental sanitation practices. In particular, animals and contaminated or untreated water have been implicated as potential sources of *H. pylori* infection. The possibility that *H. pylori* may be a zoonosis first arose following reported findings that the prevalence of *H. pylori* infection in abattoir and meat workers had significantly increased as compared to other people not involved in handling...
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animals or animal products(43). Dore et al have also reported a positive association between the prevalence of \textit{H. pylori} in Sardinian shepherds and contact with sheep and sheep dogs(44). In their study, 98% of shepherds were shown to be infected with \textit{H. pylori}, a prevalence that was significantly higher than those of other family members who did not have regular contact with sheep (73%). Moreover, the isolation of \textit{H. pylori} from the stomach of an entire colony of pathogen-free animals suggested that animals represent an important reservoir of \textit{H. pylori}(45).

Similarly, unhygienic sources of water are important routes of \textit{H. pylori} transmission; our study observed that consumption of well water or tap water had a near significant association to \textit{H. pylori} infection which was consistent to earlier findings in healthy Nigerian children with poor socio-economic status(46) and other developing countries(28).

The use of $^{13}$C urea breath test and other invasive methods are cumbersome, expensive, relatively unavailable tests in many resource limited countries where infrastructure posed a challenge(47), but noninvasive methods such as stool antigen test has proven to be effective in children and immune-compromised individuals indicating low prevalence compared to serological tests with higher outcomes. However, \textit{H. pylori} infection is mostly acquired during childhood which persists in adulthood, but it is also suggestive that the HIV-infected patients studied might have been exposed to the pathogen early in life and this infection could be lost on account of several healthy life style and ART treatment after acquired HIV-1. Similarly, the \textit{H. pylori} gastric load might be decreased in the HIV-1 patients due to interplay of CD4 cell count, gastric mucosal colonization by other pathogens and indiscriminate use of either antibiotics or proton-pump inhibitors (PPIs) which is known to modify the mucosal environment. The use of antibiotics for treatment or prophylaxis on initiation of HIV-1 infected patients and possible cofounders could suggest low prevalence and or misdiagnosis of \textit{H. pylori}(48).

CONCLUSION

The prevalence of \textit{H. pylori} infection in HIV-1 infected patients on suppressive therapy was low using HpSA ELISA stool antigen test which is effective in detecting true presence of the antigen and acute infection. However, an interesting finding on the association of marital status, housing accommodation, source of drinking water and signs of dyspepsia was observed. Other factors such as; sanitary practices and pet keeping were associated with \textit{H. pylori} infection though not statistically significant, but suggests involvement of poor socio-economic status of \textit{H. pylori} co-infected with HIV-1 infected individuals. Though the association of \textit{H. pylori} through poor sanitary practices and pet keeping has been documented in this study, therefore attention should be given to fecal-oral transmission of \textit{H. pylori} infection as well as other possible routes of transmission in order to reduce infection among low social class of people. There is need for more studies of large sample sizes to be carried out using stool antigen test because they are simple, non-invasive, relatively inexpensive and reliable assays in the diagnosis of \textit{H. pylori} infection.

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CONFLICT OF INTERESTS

Authors have declared that no conflicting interests exist.

REFERENCES


