The Use of *Helicobacter Pylori* Stool Antigen Test for the Diagnosis of *Helicobacter Pylori* in Lagos, Nigeria

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ABSTRACT

Objectives: *This study was carried out to screen the use of* Helicobacter pylori *stool antigen (HpSA) tests for diagnosis and monitoring of* H pylori *in Nigeria.*
Methods: *Seven hundred and forty participants were enrolled after informed consent was obtained, while 83 came back for a post-eradication test. The stool samples were taken from the patients at endoscopy and tested for HpSA.*
Results: *The proportion of patients that were positive at the pretest, 520 (70.3%) was significantly higher (Fisher's exact* p = *0.001) than those positive at the post-test, 44 (53%).*
There was a significant difference (F = 4.106, p = *0.043) between the mean age of those that came for the pretest (40.0 ± 14.5 years) and those that came for the post-test, 43.6 ± 11.6 years. More males than females had the tendency to come back for a post-eradication test.*
Conclusion: *Although potential bias was introduced during this study, HpSA using monoclonal antibody could still be used for diagnosis and monitoring of* H pylori *in Nigeria.*

Keywords: *Helicobacter pylori*, stool antigen test

El uso del test de Antígeno en Heces para el Diagnóstico de la Infección por *Helicobacter pylori* en Lagos, Nigeria

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RESUMEN

Objetivos: *Este estudio se llevó a cabo con el propósito de examinar el uso del test de antígeno en heces (HpSA) para el diagnóstico y monitoreo de* Helicobacter pylori *en Nigeria*
Método: *Tras obtener su consentimiento informado, se enrolaron ciento cuarenta participantes, mientras que 83 regresaron para un test de post-erradicación. Las muestras de heces fueron tomadas de pacientes en endoscopia e investigadas en busca de HpSA.*
Resultados: *La proporción de pacientes que resultaron positivos en el test previo, 520 (70.3%) fue significativamente mayor (Test exacto de Fisher* p = *0.001) que la de los que resultaron positivos en el test posterior, 44(53%). Hubo una diferencia significativa (F = 4.106,* p = *0.043) entre la edad promedio de los que vinieron al test previo (40.0 ± 14.5 años) y la de aquellos que vinieron al test posterior, 43.6 ± 11.6 años. Más varones que hembras mostraron tendencia a regresar al test de post-erradicación.*
Conclusión: *Aunque un sesgo potencial fue introducido en este estudio, HpSA con anticuerpos monoclonales podría todavía usarse para el diagnóstico y monitoreo de H pylori en Nigeria.*

Palabras claves: *Helicobacter pylori*, prueba de antígeno en heces

West Indian Med J 2011; 60 (1): 33

INTRODUCTION

Helicobacter pylori is a causative agent of gastritis and peptic ulcer disease, and is a risk factor in the development of gastric cancer (1). It is also the causative agent of mucosa associated lymphoid tissue lymphoma. In fact, the International Agency for Research in Cancer (IARC), an arm of

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WHO, has classed *H pylori* as a class I carcinogen, the highest rank given to a cancer causing agent.

H pylori infection is mainly acquired in childhood most often in the developing countries due to the influence of socio-economic factors on *H pylori* prevalence (2–3).

H pylori infection can be diagnosed by two main methods. Invasive tests that require endoscopy and non-invasive or minimally invasive tests that do not require endoscopy. The invasive tests include, CLO tests, culture, histology, direct gram stain and PCR-based methods while the non-invasive tests include serology, *Helicobacter pylori* stool antigen (HpSA) test and urea breath test (UBT). The choice of test depends on the clinical circumstances surrounding a particular patient. Invasive tests of which culture is a part is very expensive and although culture is considered the gold standard it is not often used for the detection of *H pylori* in developing countries. However, in cases where treatment failures or persistent infections are to be monitored, it is unavoidable.

The availability of reliable non-invasive tests has gained importance with increasing problems of treatment failure and subsequent eradication and this has made these tests more relevant for post-therapy assessment (4). Although the urea breath test (UBT) is the gold standard used for the primary diagnosis and monitoring of *H pylori* eradication (5), the test is expensive and not affordable or easily available (due to cost) to a majority of people from the developing world. The serological test, although cheap, is not reliable for monitoring *H pylori* eradication but only useful for epidemiological purposes as antibody titres fall very slowly after successful eradication. The HpSA test has been reported to be as reliable as the UBT for diagnosis as well as for monitoring *H pylori* eradication albeit at a lower sensitivity (6–8). The HpSA is not as expensive as the UBT and since most people in the developing countries cannot afford the cost associated with invasive tests or UBT, the aim of this project was to evaluate the HpSA kit as a reliable diagnostic and monitoring tool for *H pylori* eradication.

SUBJECTS AND METHODS

A total of 740 outpatients who complained of gastroduodenal disorders were enrolled in this study from Feb 2005 – Feb 2008. Convenient sampling was done as this was dependent on the patients that had money to pay for endoscopy and were originally scheduled for endoscopy by a gastroenterologist. The patients underwent endoscopy and stool antigen tesing using the HpSA test kit (HpSA, Meridien UK) to screen for *H pylori.* The post treatment assessment of *H pylori* status consisted of only HpSA and this was done four weeks after receiving antibiotic treatment.

The exclusion criteria were previous use of NSAIDs, PPIs, antibiotics within four weeks prior to endoscopy or HpSA test. Informed consent was obtained from the patients prior to participation in this study.

A total of 740 stool samples were tested for *H pylori* using the stool antigen test in the molecular biology labora-tory. The stool test was carried out according to the manufacturer's instructions. Briefly, one ml of sample diluent was transferred into a vial and a stool sample proportion was added into the vial with shaking using a wooden applicator to suspend. This was then vortexed for 5 minutes after which it was left for 3 minutes to allow the solid particles to settle and 500 µl of the supernatant was transferred to another vial. The reaction strip was then dipped into the second vial with the arrow pointing to the bottom and results read after 5 minutes in the white area. A positive result was recorded when in addition to a blue band, pink/red band was seen across the white central zone of reaction while a negative result showed only one blue coloured band. The stool test used was the rapid strip HpSA (Meridien, Bioscience Europe) that utilized a monoclonal anti-*H pylori* antibody.

The treatment regimen used for eradication of *H pylori* for all positive patients was 20 mg omeprazole twice daily, 500 mg clarithromycin twice daily and 1g amoxycillin twice daily for 10–14 days. Patients were screened for post-treatment eradication, four weeks after completion of therapy. Statistical analysis was done using group statistics, chi-square and Fisher's exact test.

RESULTS

A total of 740 patients comprising 357 males and 383 females (age range 6 – 86 years) were screened in this study. Out of these 740 patients, only 83 came back for a post-treatment eradication test with HpSA.

A total of 695 patients were endoscopically diagnosed with duodenal ulcer and gastritis respectively, while 40 patients had gastric ulcer and only two had gastroesophageal reflux. In three cases, the patients complained of itching and the endoscopic findings were normal.

There was a significant difference ($F = 4.106$, $p = 0.043$) between the mean age of those that came for the pretest *ie* before antibiotic therapy (40.0 ± 14.5 years) and those that came for the post-test *ie* after antibiotic therapy, 43.6 ± 11.6 years. The proportion of males increased from 48.2% in the pretest to 57.8% in the post-test, indicating that the males had a higher tendency to come back than the females, although this was not statistically significant (Fisher Exact $p = 0.106$).

The proportion of patients that were positive at the pretest, 520 (70.3%) was significantly higher (Fisher's Exact $p = 0.001$) than those positive at the post-test, 44 (53%). All patients that presented with itching were positive for *H pylori*. The study did not analyse patient compliance to the drugs as it was difficult to monitor the usage of the drugs by the patients.

DISCUSSION

From this study using the HpSA test, 70.5% of the patients were positive for *H pylori* and the majority had duodenal ulcer. Even the few (three) that presented with urticaria were *H pylori* positive. Although a variety of tests that are highly sensitive and specific abound for the diagnosis of *H pylori*, the test that would be most suitable in our environment in terms of cost and availability, as well as would help in the accurate diagnosis of *H pylori* would be the key. HpSA has been reported to be highly sensitive in the diagnosis of *H pylori* before and after eradication with sensitivities and specificities reaching 90–98% (9, 7). The UBT (both ^{13}C and ^{14}C) although the gold standard for non-invasive tests is usually not affordable and readily available in our environment. From a previous report by Krausse *et al* (10), the rapid HpSTAR stool antigen test was found useful for detecting infections in adults. While another study showed that the UBT with the ^{13}C had a lower specificity than the monoclonal stool enzyme immunoassay analysis (11).

Although 53% of the patients were still positive after treatment, it goes to show that some of the patients placed on a regimen that included metronidazole/amoxycillin could have been infected with *H pylori* resistant to these drugs. To buttress this fact, a previous report by Smith *et al* (12) showed high amoxycillin and metronidazole resistance amongst *H pylori* strains in Nigeria. Another reason could be as a result of patient compliance as some patients might not have taken the right dose of the drugs. The 53% still positive after treatment could be related to the smaller number of patients (83) that came back for post treatment eradication test, perhaps a bigger sample size would have reduced the percentage of positive cases. From another report by Manes *et al* (13) on assessing the accuracy of a new monoclonal stool antgen test in post eradication assessment of *H pylori* infection, the UBT was found to be most useful followed by the monoclonal stool antigen test.

However, a potential bias has been introduced in this study due to the fact that a convenient sampling method was used for the selection of patients.

Most patients could not afford to pay for a second endoscope and test. Thus, the majority of the patients did not report back for the post-treatment eradication test as well as endoscopy. The possibility still remains that either some of the patients successfully had their *H pylori* eradicated or did not have sufficient funds to come back for post-eradication tests. In Nigeria, the majority of the people do not have health insurance and only recently was health insurance introduced to government staff to cover only certain expenses. In this study, there were fewer patients that did not have their *H pylori* eradicated and the majority were males. One of the reasons could be due to patient compliance to the drugs (although the study did not look into this) and an inappropriate drug regimen as there was no culture done to find out the right regimen to use for treatment.

Helicobacter pylori infections have been suggested to play a role in the pathogenesis of a variety of dermatological diseases (14) although there is no strong evidence linking cutaneous pruritus and a variety of others. However, a study by Kandyil *et al* (15) concluded that there were some patients with refractory pruritis and active *H pylori* infection, whose pruritis resolved after *H pylori* eradiation. From this study using HpSA, three patients that complained of itching were positive for *H pylori*, the patients were lost to follow-up as they never returned for post-treatment tests.

The use of HpSA using the monoclonal antigen test could be reliable for diagnosis and monitoring of *H pylori* eradication in Nigeria, particularly as there are very few centres in the country with UBT facilities. This is also in addition to the fact that most patients cannot afford the cost of UBT test and would rather go for the cheaper HpSA. Information is ongoing to enlighten the public about the importance of coming back for a post-eradication test, but all still borders on finance.

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