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COMPARATIVE STUDY OF STOOL ANTIGEN TEST AND SEROLOGY FOR *HELICOBACTER PYLORI* AMONG NIGERIAN DYSPEPTIC PATIENTS- A PILOT STUDY

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ABSTRACT

Objective: The purpose of this study was to compare the stool antigen (SAT) and immunoglobulin G (IgG) serology tests for *helicobacter pylori* in dyspeptic patients in Nigeria, and determine their usefulness.

Method: Forty six patients with dyspepsia and age and sex-matched healthy controls had their blood and stool collected and screened for H. pylori infection using the enzyme linked immunosorbent assay (ELISA) IgG serology and SAT respectively.
Prevalence of *H. pylori was* 67.4% and 78.3%, among dyspeptics and controls respectively ((p=0.48) with the SAT while the corresponding values for IgG serology were 67.4% and 91.3%, p=0.005).

Result: Patients aged ≥50 years(8) were more positive to SAT (80%), compared with controls(13) which recorded more positivity in the age range 30-39 years (92.9%). The male gender had more positive SAT in patients (n=15, 75%) but the SAT was more positive among the female controls 22(84.6%). Controls in the age range <30 years were more positive to *H. pylori* IgG while the patients were more positive at = 30 yrs 10(100%).

Conclusion: It is concluded that SAT and IgG serology for H. pylori are both useful in diagnosis of the infection, and are fairly comparable in their ability to detect infection, even in area of high endemicity.

Key Words: Dyspepsia, stool antigen test, Helicobacter pylori, serology.

INTRODUCTION

Dyspepsia is a common presenting complaint encountered in clinical practice affecting about 40% of the adult population[1] It presents as chronic or recurrent pain or discomfort in the upper abdomen often in association with meals/hunger, belching, bloating, nausea, vomiting, early satiety and heartburn.
Knowledge about *Helicobacter pylori (H. pylori*) has gained prominence in the understanding and management of dyspepsia in recent times, the organism having been implicated in the pathogenesis of many of the gastroduodenal diseases presenting with dyspepsia.
The prevalence of *H. pylori* in developed countries is reported to be less than 60% in the normal adult population but the figure is far higher in developing countries[2,3], with a prevalence exceeding 90% even in normal individuals[4]. In South West Nigeria, prevalences as high as 88-94.5% were obtained in some studies using serological methods[4,5].
Invasive tests for isolation of *H. pylori* employ gastroduodenal biopsies for culture, urease test, histopathology and polymerase chain reaction (PCR). The non-invasive tests, however, do not require tissue biopsies, and include urea breath test (UBT), immunoglobulin G or M (IgG, IgM) serology, and lately, saliva antibody test and detection of *H. pylori* antigen in stool samples[6,7].
Serological screening, though widely used, is of limited value in the detection of current infection, especially in hyper-endemic areas like Nigeria since it may represent a current or a previous exposure to the organism. UBT detects current infection but is cumbersome, expensive and requires the use of radioactive material and highly technical equipment and manpower.
Stool antigen test (SAT) is based on detection of *H. pylori* antigen in the stool and therefore detects current infection[8]. Moreover, samples for SAT could easily be obtained by patients in the privacy of their home, obviating the need for venepuncture with attendant potential risk of infection to the patient and phlebotomist and analysis could be carried out in most routine laboratories within hours. In view of the high prevalence of *H. pylori* infection, the cost, inconvenience and risks associated with gastroduodenal endoscopy and the reluctance of many patients to undergo invasive procedures, SAT might provide a cost-effective, rapid and reliable diagnosis of *H. pylori* infection as well as post-

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eradication assessment in patients with dyspepsia.
The objective of this study was to determine the prevalence of H. pylori infection among dyspeptic patients using the SAT and compare with the IgG serology as well as determine the usefulness of the SAT in an *H. pylori* endemic region of the world.

MATERIALS AND METHODS

Forty-six consecutive patients with clinical features of dyspepsia and 46 age and sex-matched apparently healthy controls were recruited from the general and medical outpatient departments of the University College Hospital (UCH), Ibadan, over a twelve-month period. Control subjects were individual volunteers with no history of gastrointestinal disease outside the hospital setting and some administrative staff of the University of Ibadan and the UCH, Ibadan.
Inclusion criteria were persistence of dyspeptic symptoms for a minimum of three months, while individuals who had taken anti-*H. pylori* eradication treatment, proton pump inhibitors or other anti-secretory drugs or antibiotics within the four weeks preceding the test were excluded. Subjects who had taken bismuth compounds in the four weeks preceding test or with clinical features of gastro-oesophageal reflux disease (GORD), were also excluded. Clinical examination was carried out on all participants and findings were carefully noted, using a data collection sheet.Five milliliters of whole venous blood was obtained from each subject and control. Sera were separated after clot retraction and frozen at -20^{0}C until analysis.
Similarly, fresh pea-sized stool sample was collected into a clean universal bottle with a wooden spatula earlier given to each participant (subject and control) and frozen at-20^{0}C .
The stool samples were analysed for *H. pylori* antigen using Premier Platinum HpSA^{TM}, enzyme immunoassay kit, while the sera were analysed for *H. pylori* IgG antibody using Premier^{TM} *H. pylori* enzyme immunoassay kit (Meridian Bioscience, Inc. Cincinnati, Ohio). Positive results are samples with spectrophotometric optical density (OD) of =0.160 at 450nm wavelength, while negative results are OD < 0.140 at same wavelength. Equivocal results are results with OD =0.140 and < 0.160.
The data collected were analysed using SPSS version 10.0 (SPSS Inc. Chicago Illinois). Means were expressed as mean ±SD. Categorical variables were compared with Pearson Chi-square, while means were compared with 't' test. The equality of proportions positive between patients and controls was investigated using the two-sample test of binomial proportions. Significant P value was specified at ≤0.05.

RESULTS

Forty-six subjects (20 males, 26 females) and 46 controls (20 males, 26 females) aged 18 years and above participated in the study. The mean age of subjects was 40.87±13.3 years while that of controls was 40.83±13.2 years (p=0.9).
The prevalence of *H. pylori* using both IgG serology and SAT were statistically significantly greater in controls compared with the patients. For IgG serology, 42(91.3%) controls were positive compared with 31(67.4%) among dyspeptic patients, (p=0.05). There were also more positive SAT in controls compared with the dyspeptic patients, 36(78.3%) vs. 31(67.4%) Table 1 and Fig. 1. This, however, did not reach statistical significance, (p=0.48). Equivocal result was obtained in one subject each in both patients and controls for the SAT. Tables 1 and 2.
Subjects aged ≥50 years were more positive to SAT and so were controls in the age range of 30-39 years (n=13, 92.9%), through to age 49 (10%), (Table 3). The SAT had a slightly higher proportion of positives in the male patients 15(75%) compared with the controls 14 (70%). The reverse was however the case for *H. pylori* IgG serology in which case, the controls 18 (90%) were in the higher proportion. There were more positive SAT among the female controls 22(84.6%) compared with female subjects, 16(61.5%). The differences observed in age and sex were however not statistically significant. Table 3.
Controls in the age range <30 years had more positive *H. pylori* IgG 10(100%). Males were more positive to *H. pylori* IgG among the subjects, 16(80.0%), while the females were more positive among the controls 24(92.3%). Stratified analyses, however, did not reveal age and sex as confounding variables in the relationship between dyspepsia and *H. pylori serology*. Table 4

Table 1: **Serology and Stool Antigen Test in Subjects and Controls.**

Variable	Subjects n=46	Controls n=46	Total n=92	χ^2	P-value
H. pylori IgG				8.03	0.005
Positive	31(67.4%)	42(91.3%)	73(79.3%)		
Negative	15(32.6%)	4(8.7%)	19(20.7%)		
Stool antigen test for *H. pylori*(SAT)				1.46	0.48
Positive	31(67.4%)	36(78.3%)	67(72.8%)		
Negative	14(30.4%)	9(19.6%)	23(25%)		
Equivocal	1(2.2%)	1(2.2%)	2(2.2%)		

Table 2: **Comparison of Stool Antigen Test and Serology in Subjects and Controls.**

Group			*H. pylori* IgG Positive (%)	Negative (%)	Total (%)	Test Statistic	P-value
Subjects	SAT	Positive	24(77.4)	7(46.7)	31(67.4)		0.067
		Negative	7(22.6)	7(46.7)	14(30.4)	χ^2=5.41	
		Equivocal	-	1(6.7)	1(2.2)		
Controls	SAT	Positive	35(83.3)	1(25)	36(78.3)		
		Negative	6(14.3)	3(75)	9(19.6)	χ^2=8.56	0.014
		Equivocal	1(2.4)		1(2.2)		

Figure 1: **Prevalence of *H. pylori* using *H. pylori* IgG (Hp IgG) and Stool Antigen Test (HpSA)**

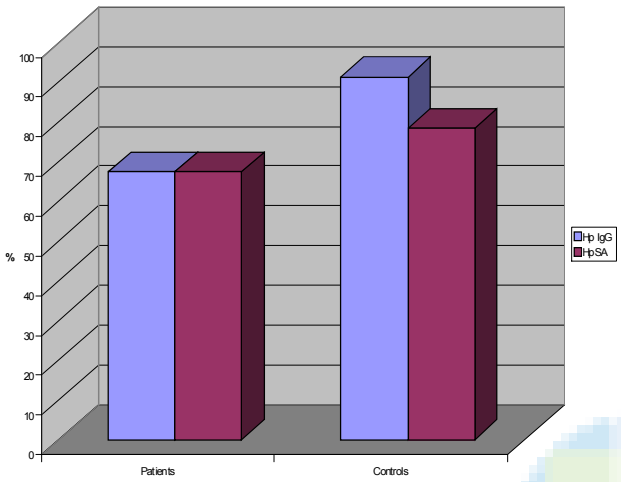

Table 3: **Demographic Characteristics and Stool Antigen among Subjects and Controls.**

			Group Dyspeptics (%)	Control (%)	χ^2	P - value
Age Group	< 30	Positive	6(60)	8(80)		
		Negative	4(40)	2(20)		
		Total	10(100)	10(100)	0.952	0.628
	30-39	Positive	9(69.2)	13(92.9)		
		Negative	4(30.8)	1(7.1)		
		Total	13(100)	14(100)	2.494	0.165
	40-49	Positive	8(61.5)	10(83.3)		
		Negative	4(30.8)	1(8.3)		
		Equivocal	1(7.7)	1(8.3)		
		Total	13(100)	12(100)	1.985	0.371
	50	Positive	8(80)	5(50)		
		Negative	2(20)	5(50)		
		Total	10(100)	10(100)	1.978	0.350
Sex	Male	Positive	15(75)	14(70)		
		Negative	5(25)	5(25)		
		Equivocal	-	1(5)		
		Total	20(100)	20(100)	1.034	0.596
	Female	Positive	16(61.5)	22(84.6)		
		Negative	9(34.6)	4(15.4)		
		Equivocal	1(3.8)	-		
		Total	26(100)	26(100)	3.870	0.144

Table 4: **Demographic Characteristics and Serology among Subjects with Dyspepsia.**

			Group Dyspeptics (%)	Control (%)	χ^2	P-value
Age Group	< 30	Positive	6(60)	10(100)		
		Negative	4(40)	-		
		Total	10(100)	10(100)	5.000	0.087
	30-39	Positive	9(69)	13(93)		
		Negative	4(31)	1(7)		
		Total	13(100)	14(100)	2.494	0.165
	40-49	Positive	9(69)	11(92)		
		Negative	4(31)	1(8)		
		Total	13(100)	12(100)	1.963	0.322
	50	Positive	7(70)	8(80)		
		Negative	3(30)	2(20)		
		Total	10(100)	10(100)	0.267	1.000
Sex	Male	Positive	16(80)	18(90)		
		Negative	4(20)	2(10)		
		Total	20(100)	20(100)	0.784	0.661
	Female	Positive	15(58)	24(92)		
		Negative	11(42)	2(8)		
		Total	26(100)	26(100)	8.308	0.009

Mantel-Haeszel X^2: Age groups= 6.35, p-value = 0.01
Sex =6.59, p-value =0.01.

DISCUSSION

This study showed a slight female preponderance (56.5% vs. 43.5% males), and a lower mean age (40.8 years) among dyspeptic patients in contrast to the pattern observed by Jones *et al*[9]. in the British population. In the British study, they observed more complaints of dyspepsia in the older age group and suggested that the older subjects were probably more concerned about their health or were afraid of more serious underlying diseases. They further concluded that this may be of advantage, in that severe diseases could be detected early and management instituted promptly. Regrettably, there was no study on stool antigen test from developing countries of the world to compare our study with.

The slight preponderance of females in our study could reflect a greater consciousness in the issue of their health, or their ready presentation in the hospital could be due to emotional/psychosomatic disorders, which tend to be commoner among the female gender. In contrast to this study, Ihezue *et al*[10] in the North-Central part of Nigeria, found that most of the dyspeptics (60%) were males and symptoms were commoner in those below the age of 40 years.

A 67.4% prevalence of *H. pylori* was obtained among the dyspeptic population in this study using both serology and stool antigen test. Most of the studies assessing stool antigen test in the diagnosis of *H. pylori* infection were directed at determining the sensitivity and specificity of the test. Hence, actual prevalence has not been a subject of serious study and scrutiny in studied populations. Rather, positives and negatives were interpreted with respect to a pre-determined gold standard. However, a study of SAT carried out in Turkey on 445 subjects aged 2-78 years

showed a prevalence of 36.6%[11]. Age stratification showed that the prevalence of *H. pylori* in those aged 16-78 years in that same study was 42.4%. The population in our study is smaller than in the Turkish study, and probably, a larger population study might affect the faecoprevalence obtained. It is also important to note that even though a similar kit was used in the Turkish study, a higher cut-off value was used in determining the positive cases in that study and this might account for the relatively lower prevalence in their study.

There is a dearth of case control studies in the assessment of the faecoprevalence of *H. pylori* as researchers focus on the symptomatic population. This is not surprising since various studies carried out both in adult and juvenile populations depicted good sensitivity and specificity[12,13] and generally, the prevalence of *H. pylori* (evaluated by various diagnostic methods) has often been reported as being higher in the dyspeptic group than in the asymptomatic controls[2] The faecoprevalence among dyspeptics in this study is comparable to the values obtained through other diagnostic tests. Using CLO-urease test and histology, Ndububa *et al*[14]. reported a prevalence of 73% among dyspeptics at Ile-Ife, Nigeria..

The fact that there has not been much experience with stool antigen test in contrast to serology in this environment prompted this case control study. It is surprising to note that the faecoprevalence of *H. pylori* is higher among controls compared with dyspeptic patients in this environment, though not statistically significant (78.3% vs 67.4%, p=0.48). This result was corroborated by a similarly higher seroprevalence among the same healthy control population, and this is statistically significant (91.3% vs 67.4%). The sero- and faeco-prevalences in the dyspeptic subjects are similar whereas the seroprevalence in the control is higher than the faecoprevalence. This is not unexpected when one considers that an individual may possess antibody to *H. pylori* subsequent to previous exposure, even though he may not be currently infected with the organism.

A seroprevalence of 91.3% among controls is comparable to the 92.7% obtained by Otegbayo *et al*[4]. in the same part of the country. The relatively lower seroprevalence among dyspeptics in our study may be due to antibiotic abuse, a common practice in this part of the world. Also worth considering is the fact that patients with functional dyspepsia usually constitute the larger percentage of dyspeptic cases and such patients are known to have more emotional/psychosomatic complaints and consequently more frequent hospital visits.

In a random serological survey conducted by Holcombe *et al*[15]. in the Northern part of Nigeria, 228 of 268 (85%) subjects were seropositive for *H. pylori*, yet only 58 (25.4%) of them had symptoms of dyspepsia. As observed in such earlier studies conducted in this part of the world, in addition to the high prevalence of *H. pylori* among controls, the cause of dyspepsia here may be of multifactorial origin[16,17,18]. This may be the likely reason why *Helicobacter pylori* has attracted the eponym of an African enigma.

A closer look at the sero- and faeco-prevalences in dyspeptics, reveals a high degree of concordance. On the strength of this, it can be deduced that the stool antigen test and IgG serology can give a fair representation of the true prevalence of *H. pylori* in dyspeptics in Nigeria. In the control group, however, the significant disparity in the sero- and faeco-prevalences may not allow for interchangeability of the two tests. Based on the principle behind the stool antigen test, it can be concluded that the actual prevalence of *H. pylori* in controls is 78.3%.

There is no significant difference in the faecoprevalences in the various age groups among the dyspeptics unlike in the Turkish study[11] in which a steady increase with age was noticed. In addition, there is neither a significant age preponderance among the controls nor any significant sex predilection among both dyspeptics and controls in this study. It does not appear that a particular gender increases the risk of *H. pylori infection.*

It is concluded that the SAT, apart from being non-invasive and easy to carry out, is useful and comparable to the IgG serology in determining the prevalence of *H. pylori* in dyspeptic patients in Nigeria. A larger community-based study with information on strains of *H. pylori* and local cut-off values is suggested, to further evaluate the usefulness of these tests and compare with histology, presently rated as the gold standard.

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