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RESEARCH ARTICLE

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Faecal Antigen and Serology Tests detect *Helicobacter Pylori*

Rabiu Adamu Tsakuwa¹, Muhammad Dauda Mukhtar², Adamu Alhaji Samaila³,
Abdulkadir Rabiu Salisu⁴, Isa Yunusa⁵, Nasiru Magaji⁶, Ahmed Muhammed⁶,
Isa Abubakar Aliyu⁷, Idris Musa Gama⁸.

¹Department of Biological Sciences, Sule Lamido University, Kafin Hausa, Jigawa Nigeria

²Department Microbiology, Bayero University P.M.B. 3011, Kano, Nigeria

³Departments of Medicine, Bayero University P.M.B. 3011, Kano, Nigeria

⁴Department of Environmental Science, Kaduna Polytechnic, Kaduna, Nigeria

⁵Department of Biochemistry, Kano University of Science and Technology, Wudil, Kano, Nigeria

⁶Department of Microbiology, Aminu Kano Teaching Hospital, Kano, Nigeria

⁸Department Biological Sciences, Bayero University, P.M.B. 3011, Kano, Nigeria

Correspondence email: rabiudamu2@yahoo.com

ABSTRACT

Helicobacter pylori is commonly associated with peptic ulcer cases as it, infects at least half of the world's population. *H. pylori* infection is typically acquired in childhood and persists chronically, probably continuing in the stomach throughout life. This research is aimed at comparing the efficacy of the faecal antigen tests with serology in detecting *H. pylori* in suspected peptic ulcer patients. Three hundred and seventy nine stool samples and equal number of blood samples were tested for antigens and antibodies to *H. pylori* respectively from 208 males and 171 females of between 7- 57 years of age. Samples from one hundred and twenty six (33.2%) and 141 (37.2%) study participants were reactive to rapid faecal antigen and serology tests respectively. It is concluded that faecal antigen and serology for *H. pylori* are both effective diagnostic tools of the infection, and either one could be useful in early detection of the disease.

Keywords: *Helicobacter pylori*, faecal antigen test, peptic ulcer, serology

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a spiral or curved gram-negative microaerophilic, flagellated bacillus which has been considered as the etiological cause of gastritis, a peptic ulcer disease [1] which over centuries has infected around 50 percent of humans throughout the world [2]. This pathogen is known to induce several gastric disorders, but may also be associated with extra gastric diseases like anaemia, dyspepsia, and some immunological disorders [3].

The high infection incidence of *H. pylori* worldwide necessitates the need for individualized treatment of highly competent diagnostic methods [1]. While these methods should fulfill the common standards of clinical diagnostics such as accuracy, sensitivity and specificity. They should also be applicable in developing countries where hygiene standards and medical support are low [2]. Certainly, the costs, time, necessary equipment and human resources as

well as the availability of point of care application are important issues which must be considered in the development of such a methods [2].

The diagnosis of *H. pylori* infection can be achieved by invasive methods such as urease test and histology [4]. Recently, non-invasive diagnostic tests based on the detection of *H. pylori* stool antigen [5-6] and serology [7-8] have been developed. Stool antigen tests have been recommended by both the American Gastroenterological Association (AGA) and the American College of Gastroenterologists (ACG) as the most accurate non-invasive test for diagnosis and for confirmation of presence of *H. pylori* [9-10]. This method is highly sensitive, specific and useful for diagnosis, therapeutic monitoring, and test of eradication. Unlike stool antigen tests, serology-based methods have a sensitivity and specificity of only 90% and cannot distinguish between active and resolved infection [11]. It is useful for detecting a newly infected patient, but it is not a good test for follow-up of treated patients because the results do not indicate present infection with *H. pylori*. The antibody titer may remain elevated for a long time after *H. pylori* eradication. Furthermore, they are not recommended by either the AGA or the ACG for monitoring infection or confirming eradication of the organism [11]. Therefore, the objective of this study is to compare faecal antigen with serological methods in early detection of *H. pylori* in an area where peptic ulcer disease is endemic.

MATERIALS AND METHODS

Study Area

A prospective study was carried out at Aminu Kano Teaching Hospital which was the most frequently attended hospital by the peptic ulcer patients in Kano state and environs; with a global location of between latitude 11° 30' north of the equator and between longitudes 08° 30' east of the Greenwich Meridian [12].

Study Subjects

A total of one hundred and seventy nine suspected peptic ulcer patients attending Aminu Kano Teaching Hospital participated in the study.

Inclusion criteria: All participants that showed signs and symptoms of peptic ulcer disease.

Exclusion Criteria: All participants without signs and symptoms of peptic ulcer disease.

Ethical Clearance

Ethical approval was obtained from the ethical committee of Aminu Kano Teaching Hospital on 21st December, 2010. Patients enrolled in this study had consented to take part in the study. Demographic data as well as possible risks factors of such patients were obtained using questionnaire.

Methods

Two methods were used for this study:

(a) One step rapid faecal antigen test: The FAT kit used in the study was one rapid step manufactured by Jei Daniel Biotech Corp (<http://www.jdbiotech.com>), cat number JHPAG02, manufactured 2009.

(b) Onsite *H. pylori* Ab rapid test -cassette (serum/plasma): manufactured by CTK Biotech, Inc. 6748 Nancy Ridge Drive, San Diego, CA 92121, USA.

Assay Procedure for Faecal Antigen Test and Result Interpretation

All specimens and kits (cassettes and reagents) were brought to room temperature (25 °C). Small portion of stool sample was taken using sterile applicator stick of the reagent bottle, transferred into the reagent container and shaken for few seconds. The cassette was removed from the foil pouch, reagent bottle was held upright with the tip pointing away from the test performer, and the tip was snapped off, the bottle was held vertically over the sample well of the cassette, 3 drops (120-150µl) of diluted stool samples were added to the sample well. Result was read within 15 mins [13]. A distinct pink band appearing on the Test region in addition to a pink control band indicated a positive result. Negative result was obtained when only one coloured band appeared on the control region (no apparent band on the Test region). Absence of colour on both regions indicates invalid result.

Serological Assay Procedure and Result Interpretation

The specimen (blood) and test components (cassette and reagents) were brought to room temperature (25°C) before use, the device (cassette) was removed from its packet, and the test device was placed on a clean and flat surface. The device was labelled with specimen's ID number, the pipette dropper was held vertically and filled with specimen. 1 drop (about 30-50ul) of specimen was dispensed into sample well and air bubble was avoided, 1 drop of the sample diluent was added immediately, and results were read in 15minutes [13]. Positive result was obtained when both C and T bands developed; the test indicates the presence of antibodies to *H. pylori* in the specimen. Negative result was when only the C band developed. Absence of C band indicates an invalid result.

Questions Answered by Participants (Questionnaire)

Occupation, type of settlements, marital status, presence of pets in house, history of the disease in family, consumption of raw vegetables from childhood to date, sources of water in adulthood, sources of water in childhood and living in crowd.

Statistical Analysis

Data for faecal antigen and serological test obtained in this study were analysed using Chi-square test. Values were considered significant when $p < 0.05$.

RESULTS

From 379 subjects tested, faecal antigen test showed 126(33.2%) positive and 253(67.0%) negative, while serology tested 141(37.2%) positive and 238(63%) negative (Table 1). Male participants tested more reactive to both faecal antigen 80 (21.1%) and serology 86 (23.0%) than their females 46 (12.1%) and 55 (15.0%) counterparts. Young age group 17years-26years had higher number 32 (8.4%) and 38 (10.0%) of faecal antigen and serology reactivity tests. Married group had higher reactivity to faecal antigen and serological tests 100 (26.4%) and 126 (33.3%) than the unmarried 26 (6.9%) and 15 (4.0%). Participants with tertiary education showed higher reactivity 39 (10.3%) and 43 (11.4%) to faecal antigen and serological tests than other counterparts. Self-employed participants presented higher reactivity to faecal antigen and serological tests 53 (13.0%) and 58 (15.3%) than other occupational groups. Participants from semi – urban settlement showed higher reactivity to faecal antigen and serological tests 45 (11.9%) and 56 (14.8%) than urban and rural dwellers.

Table 2 shows reactivity to faecal antigen and serological test of *H. pylori* in relation to demography. From the results, participants who consumed well water had higher reactivity 39 (10.3%) and 54 (14.3%) to faecal antigen and serology tests respectively. Subjects who lived in crowd exhibited high reactivity 97 (25.6%) and 116 (30.6%) to faecal antigen and serology tests respectively. Participants who are in possession of pets, had family history of the disease and consumed raw vegetables showed more reactivity 85 (22.4%) vs 87 (22.9%), 82 (21.4%) vs 85 (22.4%) and 126 (33.2%) vs 141 (37.2%) to faecal antigen and serological tests respectively.

Table 1: Reactivity to Faecal Antigen and Serological Test of *H. pylori* in relation to Demography

Demographic characteristics	Total No. (%)	Faecal Ag Test (%)		Serology Test (%)	
		Reactive	Non-reactive	Reactive	Non-reactive
Sex					
Male	208(54.9)	80(21.1)	128(34.0)	86(23.0)	122(32.2)
Female	171(45.1)	46(12.1)	125(33.2)	55(15.0)	116(31.0)
Total	379	126(33.2)	253(66.8)	141(37.2)	238(63.0)
P-Value					0.0657 ^{NS}
Ages					
7-16	50(13.2)	16(4.2)	34(9.0)	18(4.8)	32(8.4)
17-26	73(19.3)	32(8.4)	41(11.0)	38(10.0)	35(9.2)
27-36	83(21.9)	24(6.3)	59(15.6)	24(6.3)	59(15.6)
37-46	64(16.9)	19(5.0)	45(11.9)	22(5.8)	42(11.1)
47-56	56(14.8)	18(5.0)	38(10.0)	20(5.3)	36(9.5)
≥57	53(14.0)	17(4.5)	36(10.0)	19(5.0)	34(8.9)
Total	379	126(33.2)	253(66.8)	141(37.2)	238(63.0)
P-Value					0.4340 ^{NS}
Marital Status					
Married	329(86.8)	100(26.4)	229(60.4)	126(33.3)	203(53.6)
Single	50(13.2)	26(6.9)	24(6.3)	15(4.0)	35(9.2)
Total	379	126(33.2)	253(66.8)	141(37.2)	238(63.0)

p-Value	0.2580 ^{NS}				
Educ. Qual.					
Arabic	70(18.5)	22(5.80)	48(12.7)	20(5.3)	50(13.2)
Primary	96(25.3)	30(7.9)	66(17.4)	38(10.0)	58(15.3)
Secondary	104(27.4)	35(9.2)	69(18.2)	40(10.6)	64(16.9)
Tertiary	109(28.8)	39(10.3)	70(18.5)	43(11.4)	66(17.4)
Total	379	126(33.2)	253(66.8)	141(37.2)	238(63.0)
P-Value	0.5996 ^{NS}				
Occupation					
Civil Servant	109(28.8)	41(10.8)	68(17.9)	44(11.6)	65(17.2)
Unemployed	70(18.50)	32(8.4)	38(10.0)	39(10.3)	31(8.2)
Self employed	200(52.8)	53(13.0)	147(38.8)	58(15.3)	142(37.5)
Total	379	126(33.2)	253(66.8)	141(37.2)	238(63.0)
P-Value	0.0069 ^S				
Settlement					
Urban	154(40.6)	39(10.3)	115(30.3)	39(10.3)	115(30.3)
Semi Urban	125(33.0)	45(11.9)	80(21.1)	56(14.8)	69(18.2)
Rural	100(26.4)	42(11.1)	58(15.3)	46(12.1)	54(14.3)
Total	379	126(33.2)	253(66.8)	141(37.2)	238(63.0)
P-Value	0.0163 ^{NS}				

Key: ^{NS} indicates not significant, ^S indicates significance

Table 2: Reactivity to faecal antigen and serological test of *H. pylori* in relation to risk factors

Possible Risk Factors	Total No. (%)	Faecal Ag Test (%)	Serological Test (%)		
		Reactive	Non-Reactive	Reactive	Non-Reactive
Sources of water in child hood					
Well	106(28.0)	39(10.3)	67(17.4)	54(14.3)	52(13.7)
River	59(15.6)	24(6.3)	35(9.2)	24(6.3)	35(9.2)
Bore hole	80(21.1)	21(5.5)	59(15.6)	12(5.5)	59(15.6)
Tap	94(24.8)	24(6.3)	70(15.6)	26(6.9)	68(17.9)
Bottle	40(10.6)	18(4.8)	22(5.8)	18(4.8)	22(5.8)
Total	379	126(33.2)	253(66.8)	141(37.2)	238(63.0)
P- Value					0.0651 ^{NS}
Sources of water in adulthood					
Well	47(12.4)	40(10.6)	13(3.4)	44(11.6)	3(0.8)
River	10(2.6)	8(2.1)	2(0.5)	9(2.4)	1(0.3)
Bore hole	62(16.4)	17(4.5)	45(11.9)	21(5.5)	41(10.8)
Tap	83(21.9)	21(5.5)	62(16.4)	27(7.1)	56(14.8)
Sachet water	126(33.2)	24(6.3)	102(26.9)	22(5.8)	104(27.2)
Bottle water	51(13.5)	16(4.2)	35(9.2)	18(4.8)	32(8.7)
Total	379	26(33.2)	253(66.8)	141(37.2)	238(63.0)
P-Value					0.0001 ^S
Living in crowd					
Yes	300(79.2)	97(25.6)	203(53.6)	116(30.6)	184(48.6)
No	79(20.8)	29(7.7)	50(13.2)	25(6.6)	54(14.3)
Total	379	26(33.2)	253(66.8)	141(37.2)	238(63.0)
P- Value					0.4627 ^{NS}
Presence of pets					
Yes	279(73.6)	85(22.4)	194(51.2)	87(22.9)	192(50.7)
No	100(26.4)	41(10.8)	41(10.8)	54(14.3)	46(12.1)
Total	379	16(33.2)	253(66.8)	141(37.2)	238(62.8)
P-value					0.0550 ^{NS}
Family history of the disease					
Yes	270(71.2)	82(21.4)	188(49.6)	85(22.4)	185(48.8)
No	109(28.8)	45(11.9)	64 (16.9)	56(1.6)	53(13.9)
Total	379	26(33.2)	253(66.8)	141(37.2)	238(62.8)
P-Value					0.0416 ^{NS}
Raw vegetable consumption					
in childhood to date					
Yes	379(1.0)	126(33.2)	253(66.8)	141(37.2)	238(62.8)
No	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	379	26(33.2)	253(66.8)	141(37.2)	238(62.8)

Key: ^{NS} indicates not significant, ^S indicates significant

DISCUSSION

Helicobacter pylori infections were found in half the population of the world [14]. Its prevalence is highly variable in relation to geography, ethnicity, age, and socioeconomic factors with high prevalence in developing countries and low prevalence in the developed world [15]. This study revealed that both diagnostic tools (faecal antigen and serology tests) were effective and did not discriminate male from female study participants. Overall reactivity to both faecal antigen and serological tests were found to be 33.2% and 37.2% in our study participants and this is higher than that reported [16-17]. It is however comparable to 36.7% and 35.4% reported [8, 18-19]. These variations may be due to socio-economic status of the study subjects.

Our study further indicated that tools for diagnosis of *H. pylori* used in this study did not discriminate among ages as earlier reported [20]. Though infection may occur in early age but individuals stay asymptomatic for decades in approximately 80% of the cases before manifestation [6]. Findings from our study indicated that both diagnostic tools used for identifying *H. pylori* are comparative and are not discriminated by marriage, education attainment and settlements. This could probably be due to individuals who were more exposed to predisposing factors of contracting *H. pylori*, like eating from un-sanitized restaurants and drinking unhygienic water are more likely to get infected. Our study indicated that reactivity to faecal antigen and serological tests in detecting *H. pylori* among the occupational group showed similar outcome, with a significantly high reactivity in self-employed individuals. This was comparable with the previous work [21]

This study discovered that faecal antigen and serologic tests showed similar efficacy in detecting *H. pylori* in individuals predisposed to some possible risk factors, such as sources of drinking water, living in crowded environment, contact with pets, family history of the disease and consumption of raw vegetables.

CONCLUSION

It is concluded that faecal antigen and serology tests for *H. pylori* are both effective tools in diagnosis of the infection, and either could be useful in early detection of the disease.

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