

***Helicobacter pylori* Stool Antigen in Asymptomatic Children in Lagos State, Nigeria.**

Abstracts

Aim: *Helicobacter pylori* stool antigen (HpSAg) is associated with chronic antral gastritis and peptic ulceration among young children, probably a major transmission mechanism of fecal-oral infection among children.

Study design: to study the prevalence and associated demographic variables among school children in Lagos, Nigeria.

Place of study: Alimosho and Ajeromi Local Government Areas of Lagos state, Nigeria between months March and September 2014

Methodology: Fecal samples of 185 apparently healthy children aged between 2 and 16 years were collected by randomized stratified sampling with respective constructive and informative questionnaire. Fecal samples were analysed for *Helicobacter pylori* Stool Antigen (HpSAg) using immunoassay test kit for HpSAg.

Result: Of the 185 children surveyed, high rate of HpSAg fecal positivity was found among ages 5 to 7 (21.6%) with no association with age group ($p=0.149$, OR= 0.67, CI=0.142-0.156). Fecal positivity among household population reveal high rate of 49.3% among 6 to 7 number of people living together, significant rate of 44.1% was recorded among the artisan but no association with the number of the people living together ($p=0.004$, OR=0.0, CI= 0.003-0.040). Significant high positive rate of 46.5% was observed among population that never had water availability ($p=0.013$, OR=0.0; CI=0.010-0.015) and 73.5% prevalence rate was observed. There is significant association ($p < 0.05$) between HpSAg positivity and closeness of their kitchen and water source while no relationship was observed with household population, constant availability of water, maternal educational level, weight and gender.

Conclusion: Prevalence HpSAg among asymptomatic children is high in overcrowded households and in families with low socioeconomic standards.

Keywords: *H. pylori*, fecal, children, stool antigen, asymptomatic

1.0 INTRODUCTION

Helicobacter pylori is associated with gastric pathologies, such as gastritis, duodenal ulcers [1, 2] and gastric calcinoma [3] however there are no antigens in stool. Infection occurs mainly in childhood and shows lifelong persistence in the gastric mucosa. The prevalence of *H. pylori* (in either fresh or stored serum) is usually found by using serologic tests that detect immunoglobulin G (IgG) antibodies to *H. pylori* infection [4]. However serologic tests have some limitations. For instance, they are not only sensitive to reagents and repeated techniques, they are subject to sero-conversion and sero-reversion [5]. There is evidence that infected individuals excrete *Helicobacter pylori* in feces, and *H. pylori* surface antigens (HpSA) which have been detected in stool specimens [6, 7]. Standard of hygiene seem to play a significant role in the

prevalence of *H. pylori* as higher incidence is reported in developing countries compared with developed countries. Studies have listed lower socioeconomic status, old age [8], overcrowding, and gender [9], proximity with pets; breast feeding [10] among risk factors for infection. Fecal-oral routes have been incriminated in the transmission of *H. pylori* [11]. Evidence suggests that infection occurs in early childhood within the first 2 years of life [10] while some work had been done on *H. pylori* among children presenting with clinical conditions in hospitals by detecting antibodies to *H. pylori* in sera samples. Although, available data suggest that oral-oral and fecal-oral routes are the most likely route of transmission of *H. pylori* infection, yet its transmission is not clearly understood. *H. pylori* infection is acquired early in life and builds up. Once established, it persists into adulthood [12]. It was reported that human-to-human contact may play a significant role in its transmission [13] while mode of transmission may be mediated by breastfeeding, overcrowding, and poor hygienic practices [14].

In developing countries, there is evidence to show that *H. pylori* usually cause suppression of the gastric acid barrier by production of potent urease, allowing entero-pathogens ingested from contaminated weaning foods to gain access to the small intestine. This predisposes pediatrics to childhood diarrhea, malabsorption of essential nutrients such as vitamins C and B12, and growth failure in childhood [15,16]. Moreover, previous prevalence rate of *H. pylori* and its mortality rate cannot be over-emphasized. In 1993, Holcombe reported 69% prevalence rate among pediatric age group from the northern part of Nigeria [17], 11% rate among children who presented with dyspepsia in a tertiary health facility in Abakaliki in southeastern Nigeria [8], 14% sero-prevalence was reported from southern Nigeria by Etukudo *et al* [18] and recently 63.6% prevalence of asymptomatic *H. pylori* in apparently healthy children in Lagos, Nigeria [14].

There is little information of report on the association between *H. pylori* infection and recurrent abdominal pain, prevalence of fecal *H. pylori* antigen and associated parent socio-demographic status among healthy children in Southwestern Nigeria. Therefore, the occurrence, prevalence and etiological factors that predispose asymptomatic Children to *H. pylori* infection were studied in Lagos State, Nigeria.

2.0 MATERIALS AND METHODS

2.1 Sample collection: Fecal samples of 185 apparently healthy children aged between 2 and 16 years were collected by randomized stratified sampling of the pediatrics population whose sample size was determined according to Hsieh *et al* [19]. Constructive and informative questionnaire were given to the parents and an ethical assent of the respective parent was obtained while additional assent forms were issued to adolescent children among the group after they had been educated on the study.

2.2 Sample analysis: Their fecal samples were analysed for HpSAg using *Helicobacter pylori* Stool Antigen (HpSAg) Rapid Test Kit Card Format (JD Biotech, China). This is an immunoassay kit coated with *H.pylori* polyclonal antibodies capable of detecting HPSA in fecal samples which is based on double antibodies chromographic lateral flow assay. Briefly, 0.5gm of fecal sample was homogenized in 1.5mL buffer in HP Sample Collection Tube, mixed gently for 2 seconds and allowed to stand for 5 minutes to let

the particles precipitate. Into the Sample 'S' region of the HpAg test cassette strip, was placed 60 uL of the treated fecal sample and allowed to react for 15 minutes. Appearance of pink colouration of both 'S' band and Control 'C' band indicate positive reaction while only pink colouration of 'C' indicate negative reaction. Absence of pink colouration in both 'S' and 'C' was termed invalid reaction.

2.3 Data analysis: Significance of risk factors associated with the occurrence and prevalence among the subjects was assessed by Chi square (χ^2) at confidence interval of 95% while the Odd Ratio of each variables was also determined at p value >0.05. Significance of the prevalence rate of HpSAg of the fecal samples among the subjects was determined by Pearson test while the correlation coefficient was calculated by the method of least squares at p<0.05.

3.0 RESULTS

Risk factor for fecal *H. pylori* prevalence: Of the 185 children surveyed, high rate of fecal positivity to HpSAg found among ages 5 to 7(21.6%), 8 to 10 years (18.4%) while males accounted for 52.9%. different age group has no association with the contraction or prevalence of fecal *H. pylori* antigen (OR= 0.67, CI=0.142-0.156). Fecal positivity in household population shows highest rate of 49.3% among 6 to 7 number of people living together while very significant rate of 44.1% was recorded among the artisan but there is no association with the number of the people living together in a room to suggest any association with the contraction of *H. pylori* with the children (p=0.004, OR=0.0, CI= 0.003-0.040). Significant high positive rate of 46.5% was observed among population that never had water availability (p=0.013, OR=0.0; CI=0.010-0.015); 34.6% among children with weight ranged 21 to 30 kg and high rate of 41.1% positivity rate was noted among the parent that always and sometimes buy food from hawkers for their children. The study population demographic survey suggested no close association with the prevalence as a risk factor.

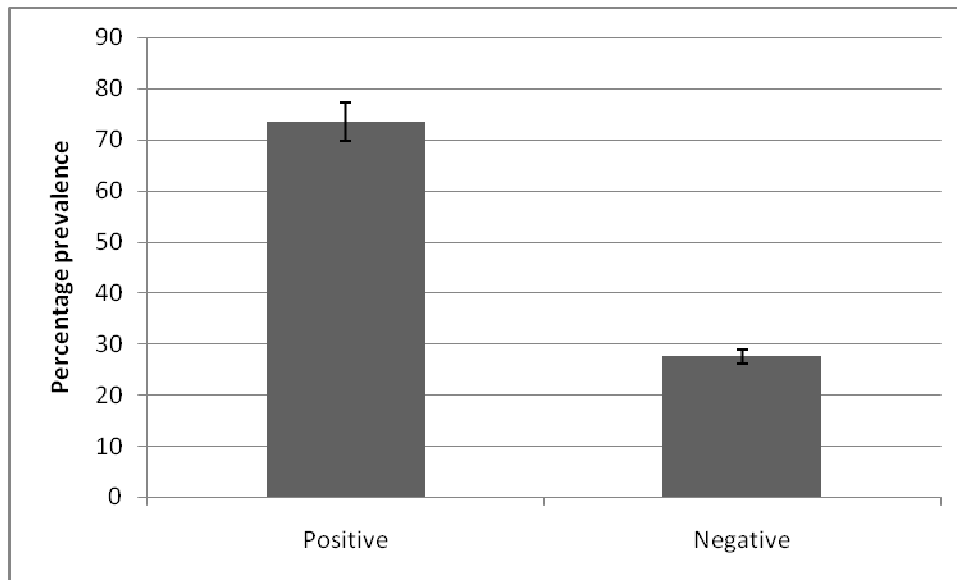
***H. pylori* Fecal antigen prevalence:** The prevalence rate of fecal positivity recorded was 73.5% among the children while lower rate of 27.6% were negative. The SEM rate was significantly high in positive cases.

Table 1; Demographic variables of the surveyed subjects with asymptomatic HpSA

Characteristic	Number positive (N=136)	Number negative (N=51) n(%)	OR (95% CI)	p-value
Age				
2-4	19 (10.3)	6 (3.2)	0.67(0.142-0.156)	0.149
5-7	40 (21.6)	12 (6.5)		
8-10	34(18.4)	13(7.0)		
11-13	25 (13.5)	16 (8.6)		
14-16	16 (8.6)	4 (2.2)		
Gender				
Male	64(47.1)	23(16.9)	0.09(0.964-0.971)	0.933
Female	72(52.9)	28(20.6)		

Household population				
2- 3	6 (4.4)	3 (5.9)	0.75(0.385-0.404)	0.395
4 -5	45(33.1)	18 (35.3)		
6 -7	67 (49.3)	25 (49.0)		
8 and above	16 (11.8)	5 (9.8)		
Parent Occupation				
Unemployed	22(16.2)	6(4.4)	0.0(0.003-0.040)	0.004
Artisan	26(19.1)	10(7.4)		
Trader	60(44.1)	15(11.0)		
Public worker	26(19.1)	20(14.7)		
Water availability				
Always	14 (7.6)	13 (7.03)	0.0(0.010-0.015)	0.013
Sometimes	34 (18.4)	19 (10.3)		
Never	86 (46.5)	19 (10.3)		
Weight (kg)				
0-10	5(3.7)	1(0.7)	0.9(0.437-0.456)	0.446
11-20	40(29.4)	17(12.5)		
21-30	47(34.6)	18()		
31-40	27(20.0)	12(23.5)		
41-50	13(9.6)	2(3.9)		
51-60	1(0.7)	1(2.0)		
61-70	1(0.7)	0(0.0)		
Eating habit				
Always buy food	56(41.4)	11(21.6)	0.0(0.0035-0.042)	0.026
Sometimes buy food	56(41.4)	31(60.8)		
Seldom buy food	15(11.1)	6(11.8)		
Never buy food	6(4.4)	3(5.9)		

Key: CI= confidence interval; OR= Odd ratio; n= number of subjects surveyed; N= total number of subjects recruited



4.0 DISCUSSION:

The prevalence of *H. pylori* infection recorded in this study is 72.4% and it is substantially higher than previous Nigerian studies [8, 18, 20]. This study shows that 14.2% of the positive children were between the ages of 2-4 years. At this age, most children will start to attend Crèche, kindergarten, public play grounds and even parties with their parents. This exposure increases their chances of being infected with *H. pylori*. The risk of exposure increases until when they become 5 - 10 when 29.9% of children would have been infected and become healthy carriers. Mothers that are Traders and Artisans have more unrestricted movements and less regulated interactions and therefore are more at risk of infections compared with parents that sedentary vocations. Therefore Artisans and Traders acquire infections more readily, become carriers and subsequently transmit the organisms to their children.

Overcrowding increases the chance of infection among the households [21]. The households that have members of 4-5 are 71.4% positive and households with 6-7 members have even a higher rate of 72.8% positivity. Although the households with 2-3 members and 8 and above seem to have low infection rate, these households are not representative. In densely populated areas such as in this study, such Households are un-common in Lagos.

The socioeconomic status of parents seems to play an important role in this study. Prevalence of 78% in children from unemployed mothers followed by 80% in children from low income traders was recorded in contrast with 56% from mothers with relatively high income. Thus standard of living may be contributory to *H. pylori* infection.

Although breastfeeding was not a focus of this study, younger children in families where more than a child participate in the study are usually negative compared with their older siblings. This could be suggestive of the protective effects of residual maternal antibodies in relatively young children and infants.

These findings confirm the work of Chak *et al* [22], which suggests that breast-feeding is protective against *H. pylori* infection. Households with regular supply of potable water would most likely have higher standard of personal and general hygienic practices. This is probably responsible for the observation in this study where families with regular water supply have lower rate of infection (10.4%) compared with 18.4% and 46.7% in households that sometimes have water supply and those that don't have water respectively. Comparing prevalence in males and females across the age groups confirms a significant relationship with more males being infected than females. Although the reason for this is yet to be understood, the result in this study is in line with the work of Etukudo *et al.* [18].

There were no significant relationship ($p < 0.05$) between infection with *H. pylori* and maternal educational level and buying food from street vendors. Also there was no statistically significant relationship between the eating habits of families that buy food from outside and being positive with *H. pylori*.

5.0 Conclusion: In Nigeria, children have been infected with *H. pylori* as early as 2 years old and have been shedding HpSAg that can be detected in their faeces. Although they are non-symptomatic children, the stool antigen are more prevalent in children aged between 5 and 13 years and prevalence decreased with age and better condition of living. Prevalence is high in overcrowded households and in families with low socioeconomic standards.

CONSENT: The Authors declared that informed assents and consent were obtained personally from all individual subjects involved in this study and any interested subject

that met the criteria of the study. They were informed of the benefit of the study and freedom to withdraw if they wish as non-invasive method of fecal collection was used.

REFERENCES

1. Gisbert JP, Pajares JM (2004) Review article: C-urea breath test in the diagnosis of *Helicobacter pylori* infection -- a critical review. *Aliment Pharmacol Ther* 20:1001-1017.
2. Farhad B., Yazdanbod A., Pourfarzi F., Sepanlou S.G., Derakhshan M. H., Reza Malekzadeh. (2012). Epidemiology of Peptic Ulcer Disease: Endoscopic Results of a Systematic Investigation in Iran. *Middle East Journal of Digestive Diseases* 4;2
3. Forman D., Newell D. G., Fullerton F. and Yarnell J. W. G., Stacey A. R., Wald N., Sitas F. (1991). Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *Brit. Med. Jour.* 1:302 (6788):1302-1305.
4. Andersen LP, Rosenstock SJ, Bonnevie O, et al.1996Seroprevalence of immunoglobulin G, M, and A antibody to *Helicobacter pylori* in an unselected Danish population. *Am J Epidemiol.* 143:1157-64.
5. Brown L M (2000). *Helicobacter pylori* – Epidemiology and Routes of Transmission. *Epidemiologic Reviews* Vol. 22. No. 2
6. Athanasios A, Michael T, Stauros S,(1998). Rapid urease test is less sensitive than histology in diagnosing *Helicobacter pylori* infection patients with non-variceal upper gastro-intestinal bleeding. *Am. J. Gastroent.* 93:1914-1918.
7. Leal Y. A., Cedillo-Rivera R., Simon J. A., Velazque J. R., Flores L. L., Torres J. 2011. Utility of stool sample-based tests for the diagnosis of *Helicobacter pylori* in children. *J. Pediatr. Gastroentol. Nutri.* 52(6). 718-728
8. Ugwuja and Ugwu 2007 Ugwuja E. I. and Ugwu N. C. 2007. *Helicobacter pylori* In Uninvestigated Dyspepsia In Primary Cares In Abakaliki, Nigeria. *Online J Health Allied Scs.* 1:4
9. Khalifa et al., 2010 Khalifa M. M., Sharaf R. R. and Aziz R. K. 2010. *Helicobacter pylori*: a poor man's gut pathogen *Gut* 2:23 – 12

10. Rothenbacher D., Inceoglu J., Bode G. and Brenner H 2000. Acquisition of *Helicobacter pylori* infection in high risk population occurs within the first two years of life. *J. Pediatr.* 136; 744-748
11. Tanih N. F., Ndip L. M., Clarke A. M. and Ndip R. N. 2010. An overview of pathogenesis and epidemiology of *Helicobacter pylori* infection. *Afr J Microbiol Research.* 4(6): 426-436
12. Sasaki K., Tajiri Y. Sasaki K., Tajiri Y., Sata M., Fujii Y., Matsubara F., Zhao M., Shimizu S., Toyonaga A. and Tanikawa K. 1999. *Helicobacter pylori* in the natural environment *Scand J Infect Dis.* 31(3):275-9.
13. Roma E, Panayiotou J, Pachoula J, Kafritsa Y, Constantinidou C, Mentis A, Syriopoulou V (2009) Intrafamilial spread of *Helicobacter pylori* infection in Greece. *J Clin Gastroenterol* 43: 711-715.
14. Senbanjo IO, Oshikoya KA, Njokanma OF (2014). *Helicobacter pylori* associated with breastfeeding, nutritional status and recurrent abdominal pain in healthy Nigerian children. *J Infect Dev Ctries* 2014; 8(4):448-453.
15. Vitale G, Barbaro F, Ianiro G, Cesario V, Gasbarrini G, Franceschi F, Gasbarrini A (2011) Nutritional aspects of *Helicobacter pylori* infection. *Minerva Gastroenterol Dietol* 57: 369-377.
16. Epplein M, Signorello LB, Zheng W, Cai Q, Hargreaves MK, Michel A, Pawlita M, Fowke JH, Correa P, Blot WJ (2011) *Helicobacter pylori* prevalence and circulating micronutrient levels in a low income United States Population. *Cancer Prev Res (Phila)* 4: 871-878.
17. Holcombe C, Tsimiri S, Eldridge J, Jones DM (1993) Prevalence of antibody to *Helicobacter pylori* in children in northern Nigeria. *Trans R Soc Trop Med Hyg* 87: 19-21.
18. Etukudo OM, Ikpeme EE, Ekanem EE (2012) Seroepidemiology of *Helicobacter pylori* infection among children seen in a tertiary hospital in Uyo, southern Nigeria. *Pan Afr Med J* 12: 39.
19. Hsieh FY, Daniel AB, Michael DL (1998). Statistics in Medicine. *Statisti. Med.* 17; 1623-1634
20. Smith S. I., Omonigbehin E. A., Goodluck H. A. Abdulkareem F. B., Onyekwere C. A., Agomo C., Ndububa D. A., Fowora M. A, Otegbayo J. A.,

219 ContrerasM., HaasR., Rieder G. (2008). Diagnostic methods for the detection of
220 *Helicobacter pylori* in Nigeria. Tropical Gastroenterol
221 21. Perry *et al.* 2006 Perry S., Sanchez M., Yang S., Haggerty T., Hurst P., Perez-
222 Perez G. and Julie P 2006. Gastroenteritis and Transmission of *Helicobacter*
223 *pylori* Infection in Households. Emerging Infectious Diseases 12(11).
224 22. Chak E., Rutherford G. W., Steinmaus C. (2009). The Role of Breast-Feeding in
225 the Prevention of *Helicobacter pylori* Infection: A Systematic Review. Clinical
226 Infectious Diseases 48:430–437
227