ORIGINAL ARTICLE

Prevalence of Helicobacter Pylori Infection and Its Association with Nutritional Status in Apparently Healthy Children Aged 1 – 59 months seen at a Tertiary Hospital in Southern Nigeria

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ABSTRACT

Background: Helicobacter pylori is the most common human bacterial infection, affecting approximately 50% of the world's population. Infection is typically acquired in childhood and persists into adulthood without treatment. H. pylori infection is associated with gastritis, peptic ulcer disease and gastric malignancies. Few studies have been conducted in resource-limited settings on H. pylori infection (HPI) in apparently healthy under-fives, where the burden of the infection is highest. **Method:** This cross-sectional-analytical-study was conducted at UBTH, Benin City, Nigeria, from November 2023 to May 2024. The study aimed to determine the prevalence and nutritional status of H. pylori in apparently healthy children aged 1-59 months using the monoclonal stool antigen test (SAT). **Result:** A total of 252 apparently-healthy-children were recruited for the study. Nutritional status was determined using weight-for-age, weight-for-height, length/height-for-age, and BMI for age z-scores based on the 2006 WHO growth standards. The DiaSpot® H. pylori Antigen Rapid Test Cassette was used to test for the presence of H. pylori antigens in stool samples. The prevalence of HPI was 26.6%. H. pylori infection was significantly associated with over-nutrition with a higher than normal BAZ score being an independent predictor of H. pylori positivity. **Conclusion:** the high prevalence of HPI in apparently healthy children suggests that screening for the infection may be beneficial. Early identification and follow-up for those who test positive could prevent the development of long-term complications. The study highlights the need for further research into the relationship between over-nutrition and HPI in children

Key words: Helicobacter pylori, Nutritional status, Apparently healthy children, Tertiary hospital

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Introduction

Helicobacter Pylori is the most common chronic bacterial infection in humans worldwide with 50% on the world's population thought to be affected at one time period or the other.[1–4] Prevalence rates in the range of 80 – 90% have been reported in developing countries, as *Helicobacter pylori* infection (HPI) is often acquired much earlier in childhood,[2]compared to the reported 10% of the population harbouring the infection in high-income, developed countries.[5]The main route of transmission is from person-to-person through faeco-oral or oral-oral route. Higher incidences of the infection in developing countries are ascribable to the deplorable sanitary conditions, overcrowding and poor environmental hygiene and pervading poverty in these countries. [1,2,6]

Helicobacter pylori (*H. pylori*) is a gram-negative, non-spore-forming, spirally-shaped bacterium that colonizes the epithelium of the human stomach, in particular the gastric antrum.[7] The organism was first isolated by Barry Marshall and Robbin Warren in 1982 following gastroscopies done on 100 patients.[8] *Helicobacter pylori* is capable of becoming virtually metabolically inactive, with minimal or synthesis of DNA and RNA through a conversion from spiral into coccoid forms, offering a survival advantage under harsh survival conditions.[9]Three forms of *H. pylori* are presently considered to exist, namely (in order of most to least virulent), the viable, culturable bacillary form, the viable but non-culturable (VBNC) coccoid form and the non-viable degenerative form.[10]

The bacterium is well adapted to living beneath the mucous layer attached to the surface of gastric enterocytes.[11] It possesses several adaptive features that aid its survival in acidic medium ranging from its flagella (used for motility), its ability to produce urease(used in neutralizing the acidity of the gastric medium) and adhesins which it uses to attach to gastric epithelium. For *H. pylori* infection to be established, doses of between 10^5–10^9cfu are required.[11]

H. pylori was designated a class 1 (definite) carcinogen by the International Agency on Cancer Research (IACR) a subsidiary of the World Health Organization in 1994 making it the only bacteria with such unenviable status.[1,2,12,13]Besides its role in carcinogenesis, H. *pylori* have been implicated in a number of morbidities that include: gastritis, gastric ulcers, and duodenal ulcers.

*Helicobacter pylori*infection, if undetected and left untreated, it can persist into adulthood with the associated morbidities ranging from peptic ulcer disease to carcinogenesis. Early identification of *H. pylori* infected children would aid prompt follow-up and treatment at the earliest sign of symptoms thus averting potential complications.

Conflicting reports exist as to the relationship between nutritional status of children and infection with *H.pylori*. *Helicobacter pylori* infection is acquired early in life and has been hypothesized to suppress the protective gastric acid barrier in children particularly in developing countries leading to diarrhoea, malnutrition and growth failure.[14]Additionally, it has been demonstrated that H.*pylori* impairs absorption of nutrients and vitamins in affected children.[15] The current study determined the prevalence of H. pylori infection and its relationship with the nutritional status in apparently healthy children aged 1-59 months presenting to a tertiary hospital, utilizing the stool antigen-based method.

Methodology

Study Location

The study was carried out at the Infant and Child Welfare Clinic (ICWC) and General Practice Clinic (GPC) of the University of Benin Teaching Hospital (UBTH), Benin City, Edo State. Edo State is situated in the South-south geopolitical zone of Nigeria with Benin City as its capital. It has an estimated population of 1,841,000 by 2022- a 3.31% increase from 2021 population.[16]The inhabitants are mainly farmers and civil servants.

UBTH is a multi-specialty tertiary facility and a referral centre. It is a 920-bedded hospital that provides primary, secondary and tertiary health care services to the entire Edo State and neighboring States of Delta, Ondo and Kogi.

The ICWC is situated within the Institute of Child Health and it has about 22 staffs including health personnel. It is open on weekdays between the hours of 8am-4pm. Children less than 2 years' access nutrition, growth monitoring and immunization services routinely in the facility. The clinic attendance register of ICWC showed that it catered to an average of about 34 children weekly.

The General Practice Clinic is a primary care clinic run by the Department of Family Medicine. It has 10 consulting rooms, a Staff clinic, Medical Records, 2 Nursing stations, a Laboratory and a Pharmacy unit. The clinic acts as a screening point for all cadre of patients irrespective of age. Medical fitness certificates are issued there. Children less than 10 years constitute about 10-20 % of the 500 – 550 patients seen per week. It is a common sight to see apparently healthy children accompanying their ill siblings in this clinic. An apparently healthy child in this research refers to children without any clinical signs and symptoms of illness based on history and physical examination. University of Benin Teaching Hospital has a Pediatric Gastroenterology Clinic that runs every Tuesday. It has 2 consultants, 2 senior registrars, 1 registrar and a house officer. The main laboratory in the hospital has capacity to perform urea breath test, H. pylori serology and Stool Antigen-based Test (SAT).

Study period

The study was conducted from November 2023 to May 2024

Study design

The study was a hospital-based, analytic and cross-sectional one.

Study population

The subjects were apparently healthy children aged 1-59 months on routine visits either at Infant and Child Welfare Clinic (ICWC) and General Practice Centre (GPC) of UBTH.

Sample size determination

The minimum sample size for the study was determined using the formula = z^2pq / d^2.[17]Prevalence of H. *pylori* infection amongst under-fives using monoclonal SAT in Nepalese children (18.2%).[18]with consecutive recruitment until desired numbers for the age groups were completed.

Inclusion criteria

Apparently healthy children aged 1-59 months. For the purpose of this study, this refers to children with the absence of disease based on clinical signs and symptoms, assessed by physical evaluation).

Children whose caregiver(s) or parent(s) gave consent for their participation in the study

Exclusion criteria

Children who were on proton pump inhibitors (PPIs) or who had been on PPI within the preceding 2 weeks.

Children who were on antibiotics or who had received antibiotics within the last 4 weeks.

Children who currently had any symptom of disease such as fever, diarrhoea, cough etc.

Children of non-consenting parents/caregivers.

Ethical considerations

Ethical clearance was obtained from the Ethics and Research Committee of UBTH (Reference number:ADM/E22/A/VOL, VII/14831008). Oral permissions were also obtained from the Heads of the various clinics used in the study. Participation in the study was entirely voluntary. Written informed consent was obtained from parents/guardians of the subjects by the researcher or his research assistants.

All data obtained from the questionnaire were treated with utmost confidentiality. All children who tested positive were referred to the pediatric gastroenterology clinic for follow up.

Sampling method

The calculated sample of 252 participants were grouped based on age in 5 groups namely 1-12, 13 -24, 25 -36, 37 -48 and 49 -59 months respectively i.e 252/5 = 50.4 per group. Since there cannot be 0.4 person, the first 2 groups are rounded up to 51 participants each and the last 3 groups are reduced to 50 participants each. Apparently healthyparticipants were recruited consecutively until each age band were filled up using a non-probability (quota) sampling method.This method allowed for equal representation across the age bands studied.

Data Collection

A semi-structured researcher-administered proforma was used to obtain relevant socio-demographic data and to inquire about the risk factors for HPI. The socio-economic status of the study subjects was determined using the scoring system developed by Ibadin and Akpede[19] in 2021. The indices used to evaluate nutritional status were weight-for- age, weight for length/height, length/height-for-age and BMI for age. The indices were standardized by calculating relative z-scores based on the 2006 WHO growth charts.

Statistical Analysis

Data was analyzed by descriptive and inferential statistics using SPSS for Windows software version 27. Means and standard deviations (SD) were calculated for continuous variables (e.g height, weight) while proportions were calculated for categorical variables if they were normally distributed. The strength of association between categorical variables was assessed using the Pearson Chi-squared (χ^2) test. Logistic regression analysis was used to evaluate the relationship between the nutritional factors and the occurrence of *H. pylori* infection. The Probability (p value) of less than 0.05 or 95% CI was accepted as being statistically significant in all cases.

Results

Socio-demographic and nutritional characteristics of the study participants

A total of 252 apparently healthy children were recruited for the study from ICWC and GPC. Of these, 15 could not produce stool during clinic visit and their caregiver/parent were given universal container to bring stool sample produced within 4 hours of collection. None of the 15 samples tested positive for H. pylori.

Table I: Depicts the socio-demographic characteristics of the study participants. Slightly above half of the participants were males with male: female ratio of 1.2:1. The mean age ± (SD) of the subjects was 32.33±15.91 months. One hundred and seventy-seven and sixty-two participants were from the upper and middle socio-economic class (SEC) respectively using the scoring system developed by Ibadin and Akpede in 2021. Most (96.4%) of the caregivers practiced Christianity. Majority of the participants were Bini's, Ishan's and Yoruba's making up about 70% of respondents cumulatively.

Table I: Socio-demographic characteristics of the study participants

Characteristics	Frequency n= 252	Percentage (%)
Age groups (months)		
1 –12	51	20.3
13 – 24	51	20.3
25 – 36	50	19.8
37 – 48	50	19.8
49 – 59	50	19.8
Gender		
Male	137	54.4
Female	115	45.6
Religion		
Christianity	243	96.0
Islam	9	4.0
Social Class		
Upper	177	70.2
Middle	62	24.6
Lower	13	5.2
Ethnicity		
Bini	83	32.9
Ishan	47	18.7
Yoruba	44	17.5
Igbo	24	9.5
Urhobo	19	7.5
Isoko	10	4.0
Others	25	9.9
Sewage Disposal		
Pit latrine	7	2.8
Water closet	245	97.2
Persons per Household		

<5	190	75.4
>5	62	24.6
Water source		
Borehole	48	19.0
Bottled water	37	14.7
Sachet water	159	63.1
Pipe borne water	8	3.2
Housing type		
1 room	30	11.9
2 rooms	88	34.9
3 rooms	107	42.5
4 rooms	23	9.1
≥5 rooms	4	1.6

Prevalence of Helicobacter pylori infection amongst study participants

The prevalence of H. *pylori* infection was 26.6%, with 67 out of participants testing positive for H. *pylori* using stool antigen test.

Nutritional status of study participants

Nutritional assessment (Table II) showed that 23.6% of participants were very tall and 31.8% were obese. There were no participants with under-nutrition (underweight, stunting or wasting).

Table II: Nutritional status of study participants

Measure	Category	Frequency n=252	Percentage (%)
WLHZ	Wasted	0.0	0.0
	Normal	175	69.4
	Overweight	77	30.6
LHAZ	Stunted	0.0	0.0
	Normal	192	76.2
	Very Tall	60	23.8
WAZ	Underweight	0.0	0.0
	Normal	235	93.3
	Overweight	17	6.7
BAZ	Normal	113	44.8
	Overweight	59	23.4
	Obese	80	31.8

*WLHZ – weight for length/height z-score, WAZ - weight for age z-score, LHAZ – length/height for age z-score, BAZ – BMI for age z-score

Association between nutritional indices and Helicobacter pylori positivity amongst study participants

The association between indicators of nutritional status and Helicobacter pylori status is as shown in table III. There was a statistically significant association between WLHZ and H. *pylori* positivity. Thirty-eight (21.7%) of 175 children with normal WLHZ were positive for H. *pylori* infection while 37.7% of the overweight category were positive, and the difference was statistically significant. Similarly, 22 of 113 (19.5%) children with normal BAZ tested positive for H. pylori when compared with the 14 of 59 (23.7%) and 31 of 80 (38.8%)children in the overweight and obese categories respectively. And this difference was statistically significant **(χ^2 =8.864, p=0.010**).

Table III: Association between nutritional indices and H. *pylori* status

Parameters	Helicobacter *pylori* status				
	Positive N (%)	**Negative N (%)**	**Total N=252**	**χ^2**	**p – value**
WLHZ					
Wasted	0 (0.0)	0 (0.0)	0		
Normal	38(21.7%)	137(78.3%)	175	5.430	**0.020**
Overweight	29(37.7%)	48(62.3%)	77		
LHAZ					
Stunted	0 (0.0)	0 (0.0)	0		
Normal	47(24.5%)	145(75.5%)	192	1.836	0.175
Very Tall	20(33.3%)	40(66.7%)	60		
BAZ					
Underweight	0 (0.0)	0 (0.0)	0		
Normal	22(19.5%)	91(80.5%)	113	8.864	**0.010**
Overweight	14(23.7%)	45(76.3%)	59		
Obese	31(38.8%)	49(61.3%)	80		
WAZ					
Underweight	0 (0.0)	0 (0.0)	0		
Normal	60(25.6%)	175 (74.4%)	235	1.955	0.162
Overweight	7(41.2%)	10 (58.8%)	17		

***χ^2** = chi-square,significant at p < 0.050, WLHZ – weight for length/height z-score, WAZ - weight for age z-score, LHAZ – length/height for age z-score, BAZ – BMI for age z-score

The association between WLHZ scores and H. pylori status of participants is highlighted in Table IV using chi-square analysis and adjusted residuals to detect which category was responsible for the significant association. Of the 67 participants who tested positive for H. pylori infection, a greater proportion was in the overweight category (37.2%). Being overweight was significantly associated with H. pylori positivity. This difference was found to be statistically significant (p=0.020). Those who were overweight were more than twice (adjusted residual= 2.3) likely to test positive for H. pylori than their counterparts with normal WLHZ (adjusted residual= -2.3).

Table IV: Sub-analysis of WLHZ and H. pylori status of participants

WLHZ			Positive	Negative	Total	χ^2	
	Normal	Count	38	137	175		
		Adjusted Residual	-2.3	2.3			df =1
	Overweight	Count	29	48	77	5.430*	LR=5.252
		Adjusted Residual	**2.3**	-2.3			**p=0.020**
Total		Count	67	175	252		

*LR = Likelihood ratio, df = degree of freedom, χ^2= chi-square

Sub-analysis of BAZ and *H. pylori* status of participants

The association between BAZ scores and H. pylori status of participants is highlighted in **Table V** using chi-square analysis and adjusted residuals to detect which category was responsible for the significant association**.** Those who were obese were significantly more likely to test positive for H. pylori **(adjusted residual= 2.9)** than their counterparts with normal BAZ **(adjusted residual= -2.2).**

Table V: Sub-analysis of BAZ and *H. pylori* status of participants

BAZ			Positive	Negative	Total	χ^2	
	Normal	Count	22	91	113		
		Adjusted Residual	-2.2	2.2			
	Overweight	Count	14	45	59	5.430*	df =2
		Adjusted Residual	-0.6	0.6			LR=8.864 **p=0.010**
	Obese	Count	31	49	80		
		Adjusted Residual	**2.9**	**-2.9**			
Total		Count	67	185	252		

*LR = Likelihood ratio, df = degree of freedom, χ^2= chi-square

Table V: Multivariate analysis of factors associated with H. *pylori* status amongst study participants

Parameters	OR (95% CI)	p-value
Religion	0.459 (0.106 -1.991)	0.296
Ethnicity	1.271 (0.830 -0.941)	0.277
Number of Siblings	0.986 (0.330 – 2.947)	0.999
WLHZ	0.400 (0.110 – 1.481)	0.171
BAZ	2.334(1.152 – 4.711)	***0.020**
Premastication	0.698 (0.383 – 1.250)	0.223

Discussion

The prevalence of HPI obtained in the current study was 26.6%. This prevalence was high and suggested that HPI was common in apparently healthy children younger than five years of age.Furthermore, the result has public health implications and contributes to the understanding of the global distribution of HPI. The observed prevalence in the present study was similar to the global prevalence of 26% in children aged 0-6 years reported in the meta-analysis conducted by Yuan *et al*[5] in 2022, 24.5% by Daniyan*et al1*[13]in 2020, 26.9% byEtukudo*et al*[20]in 2009 and 32.8% by Babatola*et al*[21]in 2015, in Abakaliki, Uyo and in Ile-Ife, Southern Nigeria.

The prevalence of this study was much lower than those reported by Ndip*et al*[22] in Cameroon,Agossou*et al*[23]in Benin Republic, Holcombe*et al*[24] in Maiduguri and Senbanjo*et al*[25]in Lagos Nigeria, which reported prevalence rates of 50%,54.3%, 40.4% and 75% respectively amongst the under-fives. A number of factors acting singly or in combinationmight influence the prevalence of H. *pylori* infection and therefore be responsible for the higher prevalence observed in those studies. These include method of analysis, environmental conditions such as crowding, source of drinking water, mode of waste disposal and hygienic practices.[1,5,20,21,26] For example, environmental conditions such as living in congested homes of five or more persons is known to influence the prevalence of H. *pylori* infection as reported by Hestvik*et al*[26] in Uganda, Awuku*et al*[1] in Ghana and Etukudo*et al*[20] in Nigeria, but in this study majority of the respondentsconstituting 75% of the total were from households of less than five persons which suggest that majority of the participants do not live in congested homes. This might have accounted for the lower prevalence observed in the current study. For example, about 85% of respondents in the study by Etukudo et al in Uyo, had 4 or more persons living in a household with a seropositivity rate of 84.5%. In addition, the present study used monoclonal stool antigen test as against serology employed in the quoted studies. Serology does not discriminate between past and present infections and hence might not be a reflection of the true prevalence in the studied populations.[1,22,26]Furthermore, despite using similar study asAgossou e*t al*[23] in Benin Republic, the prevalence from the present study is much lower than this study.This may be due to the hospital-based nature of this study in contrast to their community based study. Also, the lower prevalence seen in the current study compared with previous studies in Nigeriamay reflect anactual decrease in the prevalence of *H. pylori* over the years in Nigeria due to improved enlightenment and living conditions amongst the caregivers which could have positively influenced behaviors and reduced transmission of H. pylori. A trend of decreasing prevalence of *H. pylori* infection among children on a global scale, particularly in industrialized countries of the Western countries has been demonstrated largely due to improvements in living conditions and sanitation.[27]However, the prevalence of 26.6% in the present study was higher than the 18.2% reported by Mehata*et al*[18] amongst under-fives in Nepal despite both studies being conducted in developing countries and utilizing monoclonal H. *pylori* stool antigen tests. A plausible reason for this difference could be due to racial differences as the prevalence of H. *pylori* infection is known to vary within regions of same country, ethnic groups, socio-cultural practices and races.[28,29]

Nutritional status was significantly associated with *H. pylori* positivity in this study. It was observed that overweight participants using the weight for height Z-scores had a significantly higher *H. pylori* positivity. Similarly, participants who were overweight or obese using the Body Mass Index Z-score had a significantly higher *H. pylori* positivity when compared with those of normal BAZ.There is paucity of studies on the association between over-nutrition and *H. pylori* positivity in children, thus making comparison of this finding with previous works challenging. However, a meta-analysis by Baradaran*et al*[30] in adult population showed a higher risk of *H. pylori* infection in obese participants. The plausible reason for the association between over-nutrition and *H. pylori* positivity is not quite apparent but may be ascribed to the effect of *H. pylori* infection on the gut microbiota. *Helicobacter pylori* infection has been reported to alter the gut microbiota with affectation of the brain-gut axis leading to dysregulated appetite and satiety, thus predisposing to over-nutrition.[31,32]More researches, to elucidate the link between over-nutrition and increased prevalence of HPI would be required.

Conclusion

From the present study, the following conclusions can be drawn regarding HPI in apparently healthy children aged 1-59 months as seen in UBTH, Benin City, Nigeria

1. The prevalence of Helicobacter pylori infection amongst the study participant (Healthy 1-59 months old) was high (26.6%).
2. An association between over-nutrition and increased Helicobacter pylori positivity was seen wherein higher than normal BAZ was found to be an independent predictor for increasedH. pyloriinfection.

Recommendation

There is a need for further research into the relationship between over-nutrition and HPI in children

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