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Distribution of *Helicobacter pylori* signal regions of *vacA* from infected patients of Benue State University Teaching Hospital Makurdi in relation to sex and age

Yaji Mnena E *, Aernan Paulyn T. and Aondofa Janet T.

Department of Microbiology. Joseph Sarwuan Tarka University, Makurdi, Benue State, Nigeria.

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Abstract

Background: *Helicobacter pylori* is a Gram-negative bacterium which causes chronic bacterial infections such as gastritis, peptic ulcer, gastric cancers and gastric malt lymphoma. The severity of these diseases may be related to sex and age. The vacuolating cytotoxin A gene is a key virulence factor and exhibits genetic diversity most especially in its signal regions. The aim of the study was to assess the distribution of *Helicobacter pylori* signal regions of vavA from infected patients of Benue State University Teaching Hospital Makurdi in Relation to Sex and Age.

Methods: A total of 80 patients referred for endoscopy were enrolled, and gastric biopsies taken from the antrum of the patients and tested by PCR then genotyped using standard techniques to identify the signal regions of the vacA gene. Demographic information, including age and sex, was recorded for each participant.

Results: The frequency of *H. pylori* alleles of signal regions of vacA detected in biopsies showed that s1 had the highest frequency of 24 (100%) followed by s1c 22 (92%) while the least were s2 s1+s2. s1a was not detected.

Presence of subspecie genotypes was not significantly associated with sex (Chi-square=6.511; p=0.089). However, the occurrence of the different subspecie was found to be significantly associated with age (Chi-square=21.343; p=0.011).

Conclusion: The findings of this study show a relationship between *H. pylori* vacA sigma regions and demographic characteristics. The variations may be due to genetic variations, environmental factors, or a combination of both. *H. pylori* infection in the study was found not to be associated with gender but was significantly associated with age. Understanding this is crucial for discovering the complexity of *H. pylori* infections and developing targeted therapeutic strategies.

Keywords: Gastric biopsies; *Helicobacter pylori*; Genotypes; Sigma regions; vacA

1. Introduction

Helicobacter pylori (*H. pylori*) is a Gram-negative bacterium that colonizes the human gastric mucosa, causing various gastrointestinal disorders, including gastritis, peptic ulcers, and gastric cancer [1,2,3]. The major factor in the virulence of *H. pylori* is the vacuolating cytotoxin A (*vacA*) gene, known for its genetic variability, particularly in the signal (s) and middle (m) regions [4,5]. The sigma (σ) regions of *vacA* further contribute to the adaptability of the bacterium and its pathogenic potentials [6].

The increased risk of infection is especially high among those living in the developing world [2] The principal reasons for these variations may involve socioeconomic differences between populations. A lack of proper sanitation, and basic

* Corresponding author: Yaji, Mnena E

hygiene as well as poor diets and overcrowding all play a role in the overall prevalence of infection [7]. *Helicobacter pylori* has been rated as a "class one" carcinogen to the gastrointestinal tract by the World Health Organization [8]. It is in the same category as cigarette smoke is to lung cancer.

It has been suggested that up to 95% of duodenal and 70% of gastric ulcers are attributed to *H. pylori* infection [9]. Most cases occur among middle aged subjects and the highly productive age groups in societies [9]. Infection can commence early in life and are lifelong if remedial actions are not taken [8].

Prevalence of 82% has been reported in children 5-9 years, 95% in adults of middle age and 70 – 90% in older adults [7]. There is an estimate of more than 80% of Africans infected with *H. pylori* [11].

The burden of *H. pylori* infection is so much that the infected individuals live the rest of their lives taking drugs, avoiding certain foods and drinks because they believe it has no cure [12]. Although extensive research has been carried out on *H. pylori*, the research so far in Nigeria has tended to focus on its prevalence in certain parts/states and there is however paucity of information on the distribution of *vacA* sigma regions, especially in relation to demographic factors such as sex and age in Benue State, Nigeria.

The difference in the outcome of the disease may be associated with the bacteria genotypes. The present study is designed to determine the genotypes prevalent in patients in relation to sex and age.

Aim of the Study

The main aim of the study is to determine the distribution of *Helicobacter pylori* sigma regions of *vacA* from infected patients of Benue State University Teaching Hospital Makurdi in relation to sex and age.

Objectives of the Study

- To detect *H. pylori* from biopsy specimens using PCR
- To determine the different strains of *H. pylori* by genotyping.
- To investigate the relationship between genotypes, sex and age.

2. Materials and Methods

2.1 Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee of the Benue State University Teaching Hospital, Makurdi and all participants were informed of the details of the study, and they consented.

2.2 Sample Size Determination:

Sample size was calculated using Raosoft (2014) Sample Size Calculator. At 0.05 alpha level of significance, 95% confidence level and a patient population size of 99 and previous prevalence 50%, a sample size of 80 was obtained.

2.3 Sample Collection

A Consultant Gastroenterologist performed the endoscopy on the participants. Gastric biopsy samples were taken from the antrum of the patients. Tiny pieces of tissue samples were collected into sterile McCartney bottles containing Brain Heart infusion broth with 1.5% glycerol and stored in the freezer at -20°C within 2 hours of collection until transported to Safety Molecular Pathology Laboratory, Enugu in ice packs for analysis.

2.4 Genomic DNA (gDNA) Extraction

The Genomic DNA was extracted using Relia Prep g DNA miniprep kit (Promega, Southampton, UK).

The 2 ml tubes to be used were selected and labeled, and 200 µl of specimen were placed in the tubes. Proteinase K (25 µl) was dispensed into the tubes and the content mixed by vortexing for one minute to destroy other proteins and release the bacteria. Lysis buffer (200 µl) was added and mixed by vortexing for 10 seconds and incubated at 56 ^{0}C for 10 minutes in a water bath to help the buffer work maximally. Binding buffer (250 µl) was added to each tube and mixed for 10 seconds. Spin columns were selected, labelled, and placed into collection tubes. The lysates were transferred into the corresponding spin columns which had silica membrane and the binding buffer to help release DNA and to adsorb to the silica membrane. The columns were centrifuged (14000 revolutions per minute (rpm)) and the flow through

discarded and new collection tubes inserted. Column wash buffer (500 µl) was used to wash the columns three times and centrifuged for 3 minutes at 14000 rpm. The flow through discarded at each step. Spin columns were placed in clean collection tubes and centrifuged (14000 rpm,1 min) to remove residual wash buffer and were placed in clean 1.5ml recovery tubes. Sterile nuclease free water (200 µl) was added into each tube and incubated at room temperature for one minute and then centrifuged (13000 rpm, 1 min). The DNA quality was checked at 260/280nm using Eppendorf Bio photometer Plus (Eppendorf, Germany). Nuclease free water was used as blank. Figure 1.2 and above were taken to be pure. The genomic DNA was labelled and used for further tests immediately and the remaining one stored in the fridge.

2.5 Multiplex PCR for Detection of *vacA* Sigma Region(s) Gene of *H. pylori*

Two different multiplex PCR mixes are used to type the signal coding region 's' region into s1 and s2 (s1 is sub-typed to s1a, s1b and s1c). Product sizes include 190 bp for s1a, 187 bp for s1b, 199 bp for s2; 286 bp for s2 and 259 bp for s1 respectively.

The primer sequences used were as suggested by [13] and are:

VA1-F	ATGGAAATACAACAAACACAC
VA1-R	CTGCTTGAATGCGCCAAAC
VA1-s2-F	ATGGAAATACAACAAACACAC
VA1-s2-R	CTGCTTGAATGCGCCAAAC
SS1-F	GTCAGCATCACACCGCAAC
SS3-F	AGCGCCATACCGCAAGAG
SS2-F	GCTAACACGCCAAATGATCC

Water was used as no template control (NTC), *E. coli* DNA as Negative Control (NC) and *H. pylori* strain from ATCC number 43526 as positive control (PC). Twelve point five microliters (12.5 µl) of 10x PCR master mix (or multiplex mix), 7.5 µl of the *primer* mix and 5.0 µl of genomic DNA was pipetted making a total reaction volume of 25 µl and put in each sample well. The Thermal Profile was set in the Eppendorf Machine as '*H. pylori* mix' as follows: 95 ^{o}C for 3 min, 95 ^{o}C for 15 sec, 52 ^{o}C for 60 sec, 72 ^{o}C for 60 sec, 72 ^{o}C for 5 min, for 35 cycles. Electrophoresis was run in 2.0 % agarose gel (with 20µl ethidium bromide placed in 0.5 x TBE buffer) at 100 V for 30 minutes and the bands viewed in UV light.

The Platinium Multiplex PCR master mix (Invitrogen, UK) was used in all multiplex reactions while all the primers were HPLC grade, synthesized by Eurofins, Germany.

2.6 Data Analysis

Data obtained from the study were analysed using Statistical Package for social Sciences (SPSS) version 20, IBM Inc. Chi square was carried out to measure association between variables. Alpha level of significance was set at 0.05.

3. Results

The frequency of *H. pylori* alleles of sigma regions of *vacA* detected in biopsies shows that s1 had the highest frequency of 24 (100%) followed by s1c 22 (92%) while the least were s2 s1+s2 (frequency=1; 4%) each. s1a was not detected (Table 1).

Presence of subspecie genotypes was not significantly associated with sex (Chi-square=6.511; p=0.089) (Table 2) However, in table 3, the occurrence of the different subspecie was found to be significantly associated with age (Chi-square=21.343; p=0.011).

Table 1 Frequency of *Helicobacter pylori* Alleles of Sigma regions of *vacA* Detected in Biopsies

Alleles	**Number (%)**
s alleles	
s1a	0 (0)
s1b	2 (8)
s1c	22(92)
s1	24(100)
s2	1 (4)
s1+s2	1 (4)

Table 2 Distribution of *Helicobacter pylori* Sigma Region(s) Subtspecies in Patients by Sex

Sex	**Genotypes detected**				**Total**
	Neg (%)	**s1+s2 (%)**	**s1b (%)**	**s1c (%)**	
Female	36(76.6)	1(2.1)	2(4.3)	8(17.0)	47(100)
Male	20(60.6)	0(0)	0(0)	13(39.4)	33(100)
Total	56(70)	1(1.3)	2(2.5)	21(26.3)	80(100)

$x^2 = 6.511$, df =1; P = 0.089

Table 3 Distribution of *Helicobacter pylori* Sigma Region(s) Subspecies in Patients by Age

Age group (years)	**Genotypes detected**				**Total**
	Neg (%)	**s1+s2 (%)**	**s1b (%)**	**s1c (%)**	
< 30	4(30.8)	1(7.7)	2(15.4)	6(46.2)	13(100)
31 – 43	21(77.8)	0(0)	0(0)	6(22.2)	27(100)
44 – 56	26(76.5)	0(0)	0(0)	8(23.5)	34(100)
> 57	5(83.3)	0(0)	0(0)	1(16.7)	6(100)
Total	56(70)	1(1.3)	2(2.5)	21(26.3)	80(100)

$x^2 = 21.343$, df =3, P = 0.011

4. Discussion

The distribution of vacA sigma regions among *H. pylori*-infected individuals in our study showed that *H. pylori* was not significantly related to a patient's gender. An implication of this result is that *H. pylori* infection is not restricted to any gender since both sexes were equally susceptible to the infection. Nevertheless, higher prevalence of *H. pylori* infection in either males or females without any significant association with sex have been reported in earlier studies [14,15] even in Nigeria by [16]. These findings contribute to the understanding of *H. pylori* genetic diversity in the specific population served by Benue State University Teaching Hospital**.**

There was a significant association between *vacA* sigma region variants and demographic factors age. These findings support the notion that genetic variability in the vacA gene may influence the clinical outcomes of *H. pylori* infection [6].

In comparisons with existing literature, some studies report similar distribution patterns of *vacA* sigma regions [17], the observed associations with age add a layer to our understanding of *H. pylori* diversity. The regional context of our

study, focusing on Benue State, Nigeria, further underscores the importance of considering geographic variations in *H. pylori* genetic makeup [18].

Understanding the distribution of *vacA* sigma regions has implications for clinical practice, diagnostic approaches and treatment strategies. Specific genetic variants may enhance the efficacy of interventions. Furthermore, our findings contribute to public health initiatives, informing targeted prevention and management strategies for *H. pylori*-related diseases in the state.

5. Conclusion

H. pylori signal regions of *vacA* from infected patients was found not to be associated with genda but was however, significantly associated with age. Our study provides a comprehensive analysis of the distribution of *H. pylori vacA* sigma regions among infected patients in Benue State University Teaching Hospital Makurdi, Benue State, Nigeria. The observed associations with age contribute to the evolving narrative of *H. pylori* genetic diversity. These findings promise for refining diagnostic and therapeutic strategies, ultimately advancing the precision of clinical interventions in *H. pylori*-related diseases.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

Ethical approval was obtained from the Health Research Ethics Committee of the Benue State University Teaching Hospital, Makurdi.

Statement of informed consent

Informed consent was obtained from all patients who participated in this study.

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