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The Frequency of *Helicobacter pylori vacA* producing genotypes in patients with gastroduodenitis and peptic ulcer from Khartoum, Sudan

LIMIA SULIMAN ALI[1]
M.Sc. Student, Microbiology Department
Faculty of Medical Laboratory Sciences AL-Neelain University
Sudan
WAFA IBRAHIM ELHAG
Associate Professor, Microbiology Department
Faculty of Medical Laboratory Sciences AL-Neelain University
Sudan
MUSTAFA ELTIGANI YASSIN
Lecturer, Faculty of Medical Laboratory Sciences
AL-Neelain University
Sudan

Abstract:

Background: *Helicobacter pylori is involved in the pathogenesis of peptic ulcer disease and associated with gastric carcinoma. It has been classified as a definite class I carcinogen by the World Health Organization. the prevalence of H. pylori infection may exceed 70% in some developing countries. An 5important virulence determinant H. pylori is vacuolating cytotoxin (VacA), which induces cytoplasmic vacuolation and produces epithelial cell damage and mucosal ulceration. This study was conducted to determine the frequency of Helicobacter pylori vacA genotypes in patients with gastroduodenitis and peptic ulcer from Khartoum, Sudan.*

Methods: *A total of fifty seven (n=57) patients with gastroduodenitis and peptic ulcer were enrolled in this study, they were collected during endoscopy by the gastroenterologists. Twelve (n=12)*

[1] Corresponding author: limiasuliman@outlook.com

Specimens positive for H pylori by presence of 16sRNA .Were analyzed using specific sets of primers for genotyping of vacA gene by PCR.

Results: *The vacA gene was present in 91.7% of the H. pylori-positive specimens. The vacA s1/m1 (33.3%) and s1/m2 (33.3%) types were the most common. The vacA s2/m2 was found in (25%), and the variant vacA s2/m1 was not detected in this study.*

Key words: *Helicobacter pylori, VacA*, gastric biopsies, PCR, Khartoum.

Introduction

Helicobacter pylori (*H pylori*) infects about half of the world population [1].

It is the causative agent of chronic gastritis (CG) and peptic ulcer disease (PUD) and a major risk factor for the development of gastric cancer (GC) and mucosal associated lymphoid tissue (MALT) lymphoma.

The virulence markers of the infecting strains such as urease, flagella, adhesins, oxidase, catalase, and vacuolating cytotoxin (*VacA*), allow *H pylori* to persist for years [2].

The vacuolating cytotoxin gene (*vac*A) encodes a vacuolating toxin that is released by *H. pylori* and that injures epithelial cells via its pore-forming ability. It contains at least two variable regions. The s region 6encoding the signal peptide) exists as s1 (including s1a, s1b, and s1c) or s2 allelic types .The m region (middle) occurs as m1 or m2 allelic types. [3,4].

Geographic differences have been detected within several of these *vacA* s1, s2, m1 and m2 regions. In general, strains containing *vacA* alleles classified as s1or m1 have been associated with an increased risk of ulcer disease or gastric cancer compared to strains containing *vacA* alleles classified as s2 or m2[5]. In North America and Western Europe infection with *H.pylori* strains containing the s1 *vacA* allele is associated with peptic ulcer disease (PUD). However in Japan, South

Korea, China and India, where s1 alleles predominate, *vacA* genotype have not been associated with a more sever clinical outcome [6].

All possible combinations of these *vac*A regions have been identified, with the exception of s2/m1. The mosaic combination of the s and m region allelic types correlates with the production of the cytotoxin and is thereby associated with the virulence of the strain[3].

Specific *cagA/vacA* genotypes correlate significantly with cytotoxin activity and peptic ulceration. Thus, the typing of *H.pylori* strains may become useful in the molecular diagnosis of gastric *H. pylori* infection [7].

In Germany, Miehlke etal found a significant association between the *H.pylori vac*A s1/ m1 genotype, cytotoxic activity and gastric cancer [2].

The aim of this study was to determine the frequency of *Helicobacter pylori* vacA alleles (s1, s2, m1, m2) in patients with gastroduodenitis and peptic ulcer from Khartoum, Sudan.

Methods

Type and duration of the study

A cross-sectional study was conducted to determine the vacuolating cytotoxin gene (*vac*A), which is the virulence factor of *Helicobacter pylori* among patients with gastroduodenitis and peptic ulcer in Khartoum state, Sudan. The study was carried out during April_November 2013, at the Military Teaching Hospital (MTH), Aneelain Diagnstic Center (ADS) and Omdurman Teaching Hospital (OTH).

Samples

A total of fifty seven (n=57) antral gastric biopsy specimens were collected during upper endoscopy from patients suffering from gastroduodenitis symptoms.

Ethical consideration was obtained from AL Neelain University-Faculty of medical lab science Research ethical Board. Data was collected using interviewing questionnaire that cover name, age and sex.

Patient's consent to participate in this study was obtained prior to enrollment. The samples were collected by gastroenterologists and were put in normal saline for further processing.

DNA Extraction

The DNA was extracted directly from biopsies with The GF-1 Tissue DNA Extraction Kit (Vivantis), using protocol of DNA Extraction from Animal Tissue, The extracted DNA Store at 4°C or -20°C.

Polymerase Chain Reaction (PCR)

For the *vacA* gene alleles analysis 2 primers specific to the particular s/m regions were used. The VA1-F/VA1-R set of primers to amplify the 259-bp (s1) or the 286-bp (s2) region of the conserved portion of *vac*A[8]. The second set of primers, VAG-R/VAG-F was used to amplify the 567-bp (m1) or the 642-bp (m2) region of the conserved portion of *vac*A [9]. And the primers sequence used were:

VacA s1/s2	VA1-F	5'-ATGGAAATACAACAAACACAC-3'
	VA1-R	5'-CTGCTTGAATGCGCCAAAC-3'
VacA m1/m2	VAG-F	5'-CAATCTGTCCAATCAAGCGAG-3'
	VAG-R	5'-GCGTCAAAATAATTCCAAGG-3'

Total reaction mixture volume was 20 µl. Using Maxime PCR Premix Kit (*i*-Tag) which content of (2.5 U i-Taq™ DNA polymerase, 2.5mM dNTPs),4µl of template DNA and 2 µl of primers (1 µl F primer, 1 µl R primer) were added into Maxime PCR Premix tubes (*i*-Tag).then distilled water added to total volume of 20 µl.

The amplification was carried out in thermal cycler according to the following conditions: initial denaturation at 95°C for 2 min, followed by 35 cycles at 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 7 min.

The final amplification products were analyzed by electrophoresis in 2% agarose gel stained with ethidium bromide, and observed under UV light.

Data was analyzed by Statistical package of social sciences (SPSS) software.

Results

In this study (57) gastric biopsies were analyzed, Twelve (12) of them were positive for *H.pylori* by presence of 16 sRNA.

Out of the (12) cases positive for *H. pylori,* 11(91.7%) were positive for *vacA* gene, and 1(8.3%) was negative, Figure (1).

Among the total of positive *vac*A gene 9 (75%) were males and 2(16.6%) were females. Table (1)

Regarding age most of positive *vac*A gene was observed among 20-30 age with rang 5 (41.7%) as shown in Table (2).

Four different *vac*A alleles were amplified by PCR. For the alleles, *vacA s1/s2* and *vacA m1/m2,* amplification bands were observed at 259/286 bp and 567/642 bp respectively, Figure(2).

The results revealed that all possible combinations of these *vac*A regions were found except s2/m1.The *vacA* s1m1 (33.3%) and s1m2 (33.3%) types were the most common. The *vacA* s2/m2 was found in (25%). (Table3). Only one sample was not amplified by both *VacA* s and m regions,

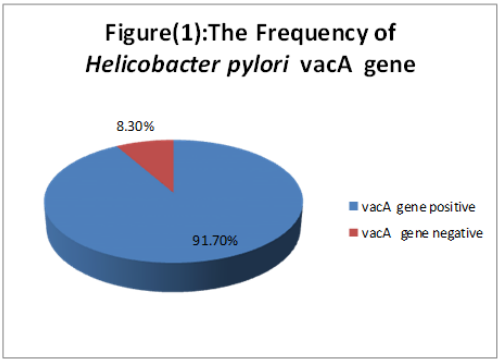

Table (1): show the presence of *vacA* gene according to sex:

Results	*vacA* gene positive	*vacA* gene negative	Total
Male	9 (75%)	1 (8.3%)	10 (83.3%)
Female	2(16.6%)	0 (0%)	2 (16.6%)
Total	11 (91.7%)	1 (8.3%)	12 (100%)

Table (2): Show the distribution of *vacA* gene according to age:

Age:	*vacA* gene positive	*vacA* gene negative	Total
20-30 years	5 (41.7%)	0 (0%)	5 (41.7%)
31-40 years	2 (16.7%)	1 (8.3%)	3 (25%)
More than 40 years	4 (33.3%)	0 (0%)	4 (33.3%)
Total	11 (91.7%)	4 (8.3%)	12 (100%)

Table (3): Show the frequency of *vacA* allele combinations:

vacA allele combinations n = 12 (100%)	Frequency
s1/m1	4(33.3%)
s2/m2	3(25%)
s1/m2	4(33.3%)
s2/m1	0(0%)
Total	11 (91.7%)

Figure (2): Electrophoresis for detection of the *vacA* gene alleles: s1, s2, m1 and m2 on 2% agarose gel. Lane M: molecular marker 100bp DNA Ladder; Lane 7: s2 allele (286bp); Lanes 4, 5, 6:s1 allele (259bp); Lanes 2, 3:m1allele (567bp); Lane1: m2 allele (642bp)

Discussion

H. pylori has been classified as a definite class I carcinogen by the World Health Organization [10].

The 91.7% prevalence of the *vacA* gene slightly lower than study report by Paniagua. (2009) which found 100% prevalence in clinical isolates of *H. pylori*[11] **.**but higher than study in a population from Northeastern Mexico which found 79.2% by Estrada .(2013) [12].

Four different *vacA* alleles amplified from samples, we found all possible combinations of these *vacA* region except s2/m1,this finding is in accordance with other studies performed in Cuban and Venezuelan population by Diana Ortiz-Princz .(2010) [3].and different from result of study in Iranian population by Fereshteh J.(2008) , which reported that s2/m1 was 8% of *vac*A alleles combinations[13] .

The predominant combination of *vac*A alleles were s1/m1and s1/m2, this findings were similar to that reported by Diana Ortiz-Princz. (2010)**,** De Gusmão. (2000), Figueiredo. (2001), Chen. 2005, Martins. (2005), Garcia.(2006), who found that s1/m1 was the most predominant combination of *vac*A alleles[3] [14] [15] [16] [17] [18].and another study in Tehran

conducted by Mohamadi. (2003) reported that s1/m2 was the predominant genotype which our finding is similar[19].

Conclusion

Infected by *H. pylori* strain with the genotype *vacA* s1/m1 and s1/m2 may increase the risk of acquiring gastroduodenitis and peptic ulcer thus it is important to diagnose the presence of these genotypes in the patients.

Competing interests

None declared

Authors' contributions

LSA, designed and conducted the study and drafted the paper. WIE, designed the laboratory procedures, and contributed in drafting the paper., MEY coordinated the laboratory quality control.

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