European Journal of Biotechnology and Bioscience
Online ISSN: 2321-9122, Impact Factor: RJIF 5.44
www.biosciencejournals.com
Volume 5; Issue 2, March 2017; Page No. 25-28

Helicobacter pylori TipA gene identified in gastric biopsies in Abidjan (Ivory Coast)

[*1] Diplo Tchépe Flore Bernadette, [2] Gbonon Mbengue Valérie C, [3] Yapo Adou Francis, [4] Guessennd Nathalie, [5] Kakou N'gazoa Solange, [6] Ouattara Aboulaye, [7] Coulibaly N David, [8] Djaman A Joseph, [9] Dosso Mireille

[1, 3, 8] Laboratory of Biochemical pharmacodynamics, Félix Houphouët Boigny, University, Ivory Coast, Côte d'Ivoire
[5, 7] Molecular biology platform of Pasteur Institute, Ivory Coast, Côte d'Ivoire
[2, 4, 9] Bacteriology-Virology Department of Pasteur Institute, Ivory Coast, Côte d'Ivoire
[6] Epidemiology Department of Pasteur Institute, Ivory Coast, Côte d'Ivoire

Abstract
Objective: To determine presence of *H. pylori* tipA gene in gastric biopsies and risk factors associated with presence of this gene in Abidjan (Côte d'Ivoire).
Material and methods: This study included 169 adult patients justifying upper endoscopy at Hospital and University Center of Cocody (Abidjan) for the period from August 2015 to January 2016. Rapid urea test was performed and tipA gene was detected by PCR. Clinical and socio-demographic information was collected from a plug investigation.
Results: Rapid urea test was positive at 58% (98/169). TipA gene was detected 58.2% (57/98). Patients with tipA gene had a female predominance of 34/57 (59.7%). Majority of the tipA gene was identified in patients aged 31-40 years (31.6%). Senior staff accounted for 18/57 (31.6%) of holders tipA gene and epigastralgias were the first indication for endoscopy, 35/57 (61.4%). More than half of patients with tipA gene (59.6%) reported having a family history of ulcer syndrome. TipA gene was observed mostly in cases of erythematous gastropathy (56.1%) and pangastropathy (24.6%).
Conclusion: high presence of tipA gene in gastric biopsies and risk factors described could contribute to characterization of *H. pylori* infection in Abidjan.

Keywords: *Helicobacter pylori*, tipA gene, gastric biopsies, Abidjan

Introduction
Helicobacter pylori (*H. pylori*) is the leading cause of development of gastric cancer in humans [1-3]. According to International Agency for Research on Cancer (IACR), it is currently the only carcinogenic bacterium of class 1 [4]. *H. pylori* exerts its pathogenesis by secretion of toxins including hemolysin, lipopolysaccharides, CagA and VacA. CagA and VacA are main virulence factors [5-7]. The persistence of H. pylori infection allows its toxins to stimulate epithelial cells to produce a large number of cytokines such as tumor necrosis factor alpha (TNF-α), interleukins 1, the 6 and 8 (IL1, IL6 And IL8), thus generating an inflammatory response [8]. Tumor necrosis factor alpha inducing protein (tipA) is a novel toxin newly discovered that probably accelerates inflammation and cancers caused by *H. pylori* [9]. This protein is coded by tip gene located at position Hp0596 of strain H. pylori 26695 and at a length of 519 base pairs (bp) [10]. It has been shown that tipA induces promotion of the tumor in vitro and in vivo [11] and that there exists on the surface of cancerous human gastric cells, a receptor called nucleolin capable of binding to tipA. The internalization of the nucleolin-tipA complex induces tumor progression and a mesenchymal epithelial, a transition in the gastric carcinogenesis process [12, 13]. The aim of this study is to determine presence of TipA gene of *H. pylori* in gastric biopsies as well as risk factors associated with presence of this gene.

Material and methods
Patients
A total of 169 adult patients ambulatory or hospitalized adult patients of both sexes justifying of a upper endoscopy at Hospital and University Center of Cocody for period from August 2015 to February 2016 was selected for the study.

Gastric biopsies
Four biopsies (2 antrum and 2 corpus) were taken from each patient and collected in sterile bottles containing 0.5-1 ml of sterile physiological water. Samples were sent to the Bacteriology-Virology laboratory of Pasteur Institute of Côte d'Ivoire under conditions of routing and within a maximum of 4 hours. Biopsies were then stored in dry tubes at -80°C.

Ethics
All patients were asked a questionnaire concerning socio-demographic data (age, sex, occupation) and medical history validated by ethics committee of Pasteur Institute of Ivory Coast. A written consent was given by each patient before endoscopy.

Rapid urea test
A biopsy of antrum and one of corus were taken and were used for rapid urea test. Biopsies were placed in a Fergusson urea-indole medium for reading within one hour. Passage from

middle of orange-yellow color to fuschia pink indicates presence of urease activity. Biopsies with positive rapid urea test were chosen for search of tipA gene. In case where antrum and corpus were positive for rapid urea test, only antrum was used for molecular biology. Also, when only one of samples was positive, only this sample was used for molecular biology.

Extraction of *H. pylori* DNA

Extraction of *H. pylori* DNA was performed according to DNA extraction protocol of NucliSENS® kit with some modifications. Biopsies were ground in 0.3 ml of 1X PBS buffer with Potter grinder into a sterile tube and then suspended in 500µl of buffer containing Tris-HCl 10 mM, EDTA 1 mM pH 8.0, Proteinase K 1 mg / Ml and incubated at 60 ° C for 24 h. DNA was extracted in 500 ml of lysis buffer containing 20 mM Tris, 2 mM EDTA, 150 mM NaCl, 1% SDS and Proteinase K 100 µg / ml for 1 h at 60°C. 1 ml of phenol-chloroform-iso-amyl alcohol mixture (25: 24: 1) was added and centrifuged at 13000 rpm for 15 min. Aqueous phase (upper phase) was collected and 1/ 10^{th} of 3M sodium acetate and 500µl of absolute ethanol were added and incubated 1 at -80°C for 1 h or overnight at -20°C. The pellet obtained is washed with 70% ethanol and dried at 65°C for 15 min. Pellet obtained is eluted in 60 µl of buffer and DNA is stored at -20°C.

Genotyping of *H. pylori* tipA gene

PCR was performed in a volume of 50 µl containing 0,75µl of each primer of 10 mM, 3µl genomic DNA, 1µl 10mM dNTPs, 3µl of 25 mM MgCl2, 5 µl of each colored and colorless 5X buffer, and 0.3 µl of Taq polymerase (Promega (R)). Amplification was performed in automaton thermocycler (Biometra® UNO II). After initial denaturation of 94°C for 5 min, amplification was 35 cycles of 94°C: 1 min; 56°C: 1mim; 72°C: 1min. Each cycle had a final elongation phase of 72°C for 7 min. Migration of PCR products was performed on 1.5% agarose gel and detection by System ™ XR GelDoc (Bio-Rad Laboratories, Hertfordshire, UK). Primers HP0596F 5'AGAGCATATGCTGCAGGCTTGCACTTGCCC-3 'and 5'-HP0596R TCTCGGATCCTACATGGCTATAGGGACTTT-3 were used to amplify DNA sequence of *H. pylori* tipA gene size of 519 bp [10]. Negative control not containing DNA was used for the quality control of amplifications.

Statistical method

Data was entered and described using software called Epi-info version 3.5.4. These data were then transcribed into Excel database thus facilitating a single, varied analysis of these data. Statistical tests were interpreted at significance level corresponding to alpha risk of 5%. Qualitative variables were compared using Pearson Chi-2 test or Fisher's exact test when one of variables was less than 5.

Results

Prevalence of H. pylori infection according to rapid urea test

Rapid urea test was positive in 98 patients with rate of 58% (98/169). There was no significant difference between the rapid urea test and the biopsy site (p> 0.05).

Prevalence of tipA gene in gastric biopsies

TipA genotype was searched for in the 98 biopsies with positive urea test. The gene was detected at 58.2% (57/98). He was absent in 41.8% (41/98). Amplification products indicating presence of tipA gene are shown in Fig 1.

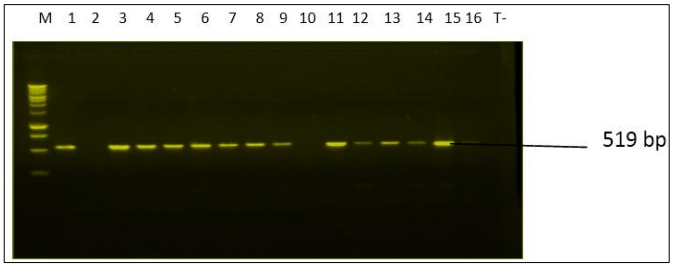

Fig 1: Genotyping of tipA gene by PCR. M: size marker, 250 bp. Line 1, 3-9, 11-16: tipA positive gene. Line 2 and 10: tipA negative gene. T-: negative control without DNA.

General characteristics of patients with tipA gene

According age, sex, profession and endoscopic indication

Patients with tipA gene had a female predominance of 34/57 (59.7%). Majority of tipA gene was identified in patients aged 31-40 years (31.6%). Senior staff represented 18/57 (31.6%) of carriers patients and epigastralgia was the first indication for endoscopy, 35/57 (61.4%) (Table 1).

Table 1: Description of the patients carrying the tipA gene according to sex, age, profession and endoscopic indication

Patients	Number	Percent(%)
Sex	Men: 23	40,3
	Women: 34	59,7
Age	20-30 years : 11	19,3
	31-40 years : 18	31,6
	41-50 years : 17	29,8
	>50 years : 11	19,3
Profession	Senior staff : 18	31,6
	Student : 5	8,7
	Employees : 9	15,8
	Unemployed : 11	19,3
	Informal sector :14	24,6
Endoscopic indication	Epigastralgia: 35	61,4
	Gastric ulcer: 7	12,3
	Non ulcer dyspepsia: 4	7,0
	Search for HTP: 4	7,0
	Halitosis: 2	3,5
	Other[1]: 5	8,8

(1) Other: dysphagia, anemia, vomiting, unilateral odynophagia, gastroesophageal reflux disease (GERD).
HTP: Portal hypertension

According family history of ulcer syndrome

More than half of patients with tipA gene, 34/57 (59.6%) admitted having a family history of ulcer syndrome.

Distribution of tipA gene according to observed endoscopic aspect

TipA gene was observed mostly in cases of erythematous gastropathy (56.1%) and pangastropathy (24.6%).

Table 2: Distribution of the tipA gene according to endoscopic aspect

Endoscopic aspect	TipA gene	
	Number	Percent (%)
Gastropathy with gastric reflux	8	14,1
Erythematous gastropathy	32	56,1
Pangastropathy	14	24,6
Other[1]	2	3,5
Normal	1	1,7
Total	57	100

(1)Other: Savary Miller's stage 2 esophagitis, congestive duodenopathy.

Discussion

H. pylori infection is a public health challenge in some developing countries due to high morbidity and mortality. In our study population, prevalence of 58% according to rapid urea test remains stable and low compared with prevalence in developing countries [14, 15]. Indeed, a real awareness of the infectious origin of gastroduodenal diseases has been observed in our populations and necessity of a therapeutic management based on anti-infectious has contributed certainly to reduce prevalence in our countries although, problem of the emergence of antibiotic resistance remains.

High prevalence of tipA 58.2% gene in our study revives notion of "African enigma" which stipulated that despite high rate of *H. pylori* infection in Africa, rate of gastric cancer was relatively low [16]. Indeed, a close involvement of tipA protein in gastric carcinogenesis has recently been described [10, 11] and many studies are performed with aim to elucidate mechanism by which tipA induces gastric cancer [12, 13]. Data on this gene are not available in Ivory Coast certainly because of its recent discovery.

Female predominance with a sex ratio of 0.67 was observed in our study. This confirms data from World Alliance against Cancer, which reported that children and young women overrepresented in populations appeared to be proportionally more affected in terms of the incidence of cancer in developing countries [17]. However, according to WHO, gastric cancer has higher rate in men than in women in Ivory Coast with major risk factors such as smoking and alcohol [18]. This contrast may be explained by lower life expectancy for men (52 years) than for women (54 years) in our current health context [18].

In study population, the mean age was 42 years with a maximum rate (31.6%) for 31 to 40 age group. This confirms that *H. pylori* contamination in developing countries occurs early in childhood and that infection is predominant in young adults [19, 20]. There would therefore be a real risk of developing gastric cancer before age 50 years. Also, patients with tipA gene whose age ranged between 41-50 years (29.8%) were approximately same percent as those aged 30-40 years. We understand why the total life expectancy of the Ivorian population is 53 years at birth [18].

In our study, senior staff accounted for 31.6% of patients with tipA gene. We expected an obvious association between socio-economic status and *H. pylori* infection due to high prevalence observed in poor populations, probably because of promiscuity and precarious hygiene that characterize them [21, 22]. However, tipA gene was more representative in persons with a good socio-economic situation. It should not be overlooked that in our current public hospital setting, the cost of conducting the endoscopy examination is high (50,000 francs CFA = $ 82), given that poverty rate in Ivory Coast is 49% and that one Ivorian on two lives on less than 1 dollar by day [23]. It is evident that people with low incomes cannot be able to perform endoscopy.

Our study showed that epigastralgia (61.4%) represented first indication of endoscopy in patients with tipA gene and 59.6% had a family history of ulcerative syndrome. This confirms primary ethiopathological role of *H. pylori* in gastroduodenal pathologies and more particularly in gastric cancer. Genetic inheritance of cancer and human-to-human transmission would be in favor of a family environment favoring infection in these patients. Recent studies have shown that there is a link between cancerous human gastric cells and tipA gene in the progression of the tumor [12, 13].

Involvement of *H. pylori* in evolution of infection to the most severe forms such as gastric cancer has been well established [24, 25]. We observed in this study that more than half of the patients with the tipA gene (56.1%) had endoscopic erythematous gastropathy. In other words, a massive presence of the bacterium in patients with gastric mucosa where persisting inflammation hence redness on the stomach mucous membranes. Indeed, these forms of gastritis are generally associated with oxydative stress generated during infection which contributes to cellular dysfunction and direct oxidative damage to the DNA [26].

Conclusion

Presence of tipA gene in gastric biopsies was high and the risk factors described were age, familial history of ulcer syndrome and endoscopic appearance. This study is the first in series that will collect data for better characterization of *H. pylori* infection and effective management of emergence of gastroduodenal diseases related to *H. pylori* in Ivory Coast.

References

1. Normark S, Nilsson C, Normark BH, Hornef MW. Persistent infection with Helicobacter pylori and the development of gastric cancer. Adv Cancer Res. 2003; 90:63-89.
2. Peek RM Jr, Blaser MJ. Helicobacter pylori and gastrointestinal tract adenocarcinomas. Nature Rev Cancer. 2002; 2:28-37.
3. Correa P. Helicobacter pylori infection and gastric cancer. Cancer Epidemiol Biomarkers Prev. 2003; 12:238s-241s.
4. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans In: IARC Monographs on the evaluation of carcinogenic risks to humans,. Schistosomes, liver flukes and Helicobacter pylori. IARC, Lyon. 1994; 61:177-240.
5. Blaser MJ, Perez-Perez GI, Cover TL. Infection with Helicobacter pylori strains possessing CagA is associated with an increased risk of developing adenocarcinoma of stomach. Cancer Res. 1995; 55:2111-2115.
6. Atherton J, Cao P, Peek R, Tumurru M, Blaser M, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of peptic vacA types with cytotoxin production and peptic ulceration. J Biol Chim. 1995; 270:17771-17777.
7. Al Qabandi A, Mustafa AS, Siddique I, Khajah AK, Madda JP. Distribution of vacA and cagA genotypes of

Helicobacter pylori in Kuwait. Acta Trop. 2005; 93:283-288.
8. Suganuma M, Okabe S, Kurusu M, Iida N, Ohshima S, Saeki Y *et al.* Discrete roles of cytokines, TNF-α, IL-1, IL-6 in tumor promotion and cell transformation. Int J Oncol. 2002; 20:131-136.
9. Suganuma M, Kurusu M, Okabe S, Sueoka N, Yoshida M, Wakatsuki Y *et al.* Helicobacter pylori membrane protein 1: a new carcinogenic factor of Helicobacter pylori. Cancer Res. 2001; 61:6356-6359.
10. Suganuma M, Kurusu M, Suzuki K, Nishizono A, Murakami K, Fujioka T *et al.* Tumor necrosis factor alpha inducing protein released from Helicobacter pylori for gastric cancer progression. J Cancer Res Clin Oncol. 2005; 131:305-313.
11. Suganuma M, Yamaguchi K, Ono Y, Matsumoto H, Hayashi T, Ogawa T *et al.* TNF-α inducing protein, a carcinogenic factor secreted from H. pylori, enters gastric cancer cells. Int J Cancer. 2008; 123(1):117-122.
12. Suganuma M, Watanabe T, Yamaguchi K, Takahashi A, Fujiki H. Human gastric cancer development with TNF-α-inducing protein secreted from Helicobacter pylori. Cancer Lett. 2012, 322(2):133-138.
13. Watanabe T, Takahashi A, Suzuki K, Kurusu Kanno M, Yamaguchi K, Fujiki H *et al.* Epithelial mesenchymal transition in human gastric cancer cell lines induced by TNF-α inducing protein of Helicobacter pylori. Int J Cancer. 2013. doi:10.1002/ijc.28582.
14. Calvet X, Ramirez Lazaro MJ, Lehours P, Mégraud F. Diagnosis and epidemiology of Helicobacter pylori infection. Helicobacter. 2013; 18(Suppl. 1):5-11.
15. Malfertheiner P, Mégraud F, O'Morain CA, Atherton J, Axon ATR, Bazzoli F *et al.* Management of Helicobacter pylori infection – the Maastricht IV/Florence Consensus Report. Gut. 2012; 61:646-64.
16. Holcombe C. Helicobacter pylori: the African enigma. Gut. 1992; 33:429-431.
17. Perrier-Bonnet S. Alliance Mondiale contre le cancer. Cancers et Afrique: le point en. 2011.
18. Organisation Mondiale de la Santé - profils des pays pour le cancer. 2014.
19. Assi C, Ndah KJ, Allah-Kouadio E *et al.* Prévalence de l'infection à Helicobacter pylori et lésions précancéreuses du cancer gastrique chez les patients souffrant d'épigastralgies chroniques. Revue Africaine de Pathologie. 2010; 9:25-31.
20. Konate A, Diarra M, Soucko-Diarra A *et al.* Gastrites chroniques à l'ère d'Helicobacter pylori au Mali. Acta Endoscopica. 2007; 37:315-320.
21. Ramanampamonjy RM, Randria MJD, Razafimahefa SH, Ratsimandisa R, Rajaonarivelo P. Séroprévalence de l'infection due à Helicobacter pylori dans un échantillon de population malgache. Bull Soc Pathol Exot. 2007; 100(1):57-60.
22. The EUROGAST Study Group. Epidemiology of, and risk factors for, Helicobacter pylori infection among 3194 asymptomatic subjects in 17 populations. Gut. 1993; 34(12):1672-6.
23. Programme des Nations Unies pour le Développement (PNUD). A propos de la Côte d'Ivoire. Bulletin info PNUD Côte d'Ivoire. 2012.
24. Suerbaum S, Michetti P. Helicobacter pylori Infection. New England Journal of Medicine. 2002; 347:1175-1186.
25. Malferthiner P, Chan FK, Mc Coll KE. Peptic Ulcer Disease. Lancet. 2009; 374:1449-1461.
26. Obst B, Wagner S, Sewing KF, Beil W. Helicobacter pylori causes DNA damage in gastric epithelial cells. Carcinogenesis. 2000; 21(6):1111-1115.