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Gene cag and other risk factors in helicobacter pylori infection in Abidjan (Ivory Coast)

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Abstract
Major role of Helicobacter pylori in the pathogenesis of gastro duodenal diseases such as gastritis, ulcers, gastric adenocarcinoma and lymphoma is well established today. Several factors are involved in virulence. Presence of Cag gene is responsible for chronic gastritis and to identify strains to be treated. The aim of this study was to determine presence of Cag gene and risk factors associated. This study included 79 adult ambulatory or hospitalized patients with clinical symptoms justifying upper endoscopy at the University Hospital of Cocody for the period from May to September 2009. Clinical and socio-demographic information was collected from a plug investigation. Detection of Cag gene was made by PCR method. The average age was 52.31 years with a minimum of 20 years and a maximum of 86 years. The most represented age group was above 50 years. Prevalence of Cag gene was investigated in 20 patients was 65% (13/20) with a male predominance of 53.8%. Unemployed accounted for 38.5% of holders of Cag gene and epigastralgia, the first such pattern endoscopy 38.5%. 46.2% of the holders of Cag gene had a family history of ulcer syndrome. Age, sex and family environment are risk factors associated with H. pylori infection in Abidjan.

Keywords: Helicobacter pylori, Cag gene, PCR, Abidjan

1. Introduction
Infection with H. pylori is manifested by inflammation of the gastric mucosa. About 10% of infected individuals will develop later peptic ulcer disease[1]. Specific genetic determinants associated with virulence of H. pylori have been described [2, 3]. The Cag gene, a region of 40 kb called "cag Pathogenicity Island" whose presence is most often associated with severe disease in humans [4, 5]. This island cag carries genes whose expression allows the formation of a type IV secretion apparatus, but also the production of CagA effector [6, 7]. Cag gene is not present in all strains but is considered a marker of the presence of the pathogenicity island on the genome of the bacterium [8]. CagA protein remains in against a useful marker to identify the strains producing cytotoxins and thus likely to be treated [9, 10]. Moreover, presence of Cag gene is responsible for chronic gastritis. In Ivory Coast, studies of Cag gene are rare. The aim of this study is to determine in symptomatic individuals, the presence of Cag gene and the risk factors associated with the presence of this gene.

1.1 Materials and Methods
1.2 Materials
This is a prospective study including 79 patients' ambulatory or hospitalized adults with clinical symptoms warranting high endoscopy at the Gastroenterology Service of the University Hospital of Cocody from May to September 2009. Four biopsies (2 antral and 2 corpus) were sent to the laboratory of Bacteriology-Virology of the Pasteur Institute of Côte d'Ivoire. Clinical information was collected through a survey sheet. Molecular detection was performed directly from gastric biopsies. Strain 01-2001 / IPCI was used as a positive control. Primers sequences used to amplify H. pylori was HpCagF/ HpCagR respectively 5'-CCATGAATTTTGATCCGTTCGG-3' and 5'-GATACAGGCAAGCTTTTGAGAGGGA-3' of size 393bp[11].

2. Methods
2.1 Rapid urease test
Rapid test of urea was performed. Two biopsies (one of the antrum and corpus) were placed in a urea-indole medium Fergusson for reading in time. The transition from the middle of the orange-yellow to fuschia pink indicates the presence of urease activity.

2.2 Gene Cag genotyping
DNA was extracted directly from one corpus and one antrum biopsy, using the extraction kit Nucli Sens® and according to the described procedure. PCR was performed in a final volume of 50 µl contained 10 µl of DNA extract, 2.5µl of each primer 10 mM, 5µl of MgCl2 25 mM, 0,4µl dNTPs 25 mM, 5µl of each colored and non-colored buffer 5X and 0,5µl Taq DNA polymerase. After heating at 94°C for 2 min, amplification was performed over 35 cycles of : 94°C for 1 min, 45°C for 1 min, 72°C for 1min and a final extension 72°C for 7 min. PCR products were resolved in 1% agarose gel stained with ethidium bromide and visualized under ultraviolet light.

2.3 Statistical method
Sphinx and SPSS software were used for data processing. Qualitative variables (sex, age, presence of Cag gene) have been described by their number and percentage. Chi2 test was

used for comparison of these variables with a coefficient of significance p <0.05.

3. Results

3.1 Prevalence of H. pylori infection

In our study, the rapid urease test was positive in 47 patients, with an overall prevalence of 59.5% (47/79) (Table 1).

Table 1: Description of the study sample according rapid test of urea

Rapid test of urea	Number	Percent (%)
Antrum		
Positive	47	59.5
Negative	32	40.5
CORPUS		
Positive	41	51.9
Negative	38	48.1

3.2 Identification of Cag gene by PCR

In our study, Cag gene was investigated in 20 patients who had their rapid urease test positive. Cag gene was found in 65% of cases (13/20). (Fig. 1).

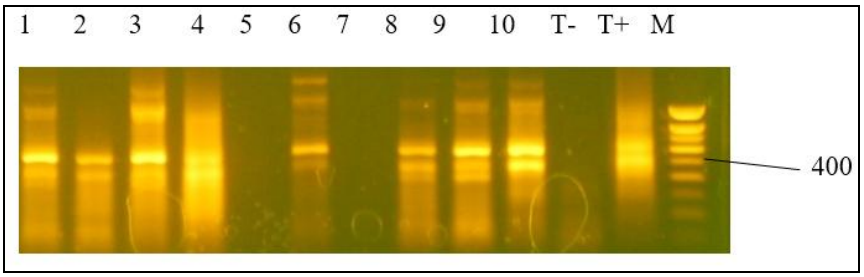

Fig 1: Cag gene PCR. Lane M: 100 bp marker. Lane T- : negative control. Lane T+: positive control. Lane 1 to 4, 6, 8, 9, 10: Cag gene positive (393pb). Lane 5 and 7: Cag gene negative. (Numbers to the right are in base pairs)

3.3 Prevalence of risk factors

According to sampling site

There was no statistically significant difference between detection at the antrum and the corpus at the same patient, OR = 0.81. However, 40% of patients had the gene in the antrum and corpus (Table 2).

Table 2: Distribution of Cag gene at antrum and corpus in the same patient

Cag detection of gene	Number	Percent (%)
Antrum+ Corpus-	1	5
Antrum - Corpus+	4	20
Antrum+ Corpus+	8	40
Antrum -Corpus-	7	35
Total	20	100

Antrum +: Presence of Cag gene in the antrum, Antrum - : Absence of Cag gene in the antrum

Corpus +: Presence of Cag ene in the Corpus, Corpus - : Absence of Cag gene in the Corpus

3.4 General characteristics of Cag gene patients

Distribution of patients according to the presence of Cag gene and sex showed a male-dominated with 53.8%. The average age was 52.31 years with a minimum of 20 years and a maximum of 86 years. The most represented age group was that of patients whose age was above 50 years. The unemployed accounted for 38.5% of holders of Cag gene. In patients of Cag gene, the epigastric pain were the first endoscopy indication pattern or 38.5%. The distribution of patients carrying of Cag gene by sex, age, profession and endoscopic indication is presented in Table 3.

Table 3: Distribution of Cag gene patients by sex, age, profession and endoscopic indication

Patients	Number	Percent(%)
Sex	Men :7	53.8
	Women :6	46.2
Age	<20 years :1	7.7
	21-30 years :1	7.7
	31-40 years :3	23.1
	41-50 years :2	15.4
	>50 years :6	43.2
Profession	Senior staff :2	15.4
	Student :2	15.4
	Employees :1	7.7
	Unemployed :3	23.1
	Informal sector :5	38.4
Endoscopy indication	Epigastralgia :5	38.4
	Non-ulcer dyspepsia :1	7.7
	Chronic anemia :2	15.4

	Hiccups	:2	15.4
	Other[1]	:3	23.1

[1] Other: lower esophageal pain, halitosis, heartburn - dysphagia.

3.5 Association between presence of endoscopic lesions and Cag gene

OR = 2.95. There is no association between presence of Cag gene and occurrence of macroscopic lesions.

3.6 Family history of ulcer syndrome

In patients Cag gene, 46.2% had a family history of ulcer syndrome.

4. Discussion

Prevalence of H. pylori infection is between 20 and 40% in developed countries; it reaches 70 to 90% in developing countries [12, 13, 14]. In our study population, prevalence of 59.5% according to rapid test of urea is underestimated compared to the prevalence noted in the developing countries [15, 16]. Indeed, the sensitivity of this test can be reduced if the bacterial density is low especially following treatment. A number of bacteria in the order of 105 is required to obtain a positive result.

This study enabled us to characterize presence of Cag gene in 65% of patients (13/20). Frequency of this gene in the world varies from 31 to 98%. The absence of this gene in certain bacteria confirms that all bacteria are not carriers. Detection of Cag gene mark presence of the pathogenicity island on the genome of the bacteria. This island contains a number of genes whose products are associated with increased virulence of *H. pylori*; such as the induction of cytokine production in gastric mucosa, resulting in severe gastritis [17]. Our results confirm those of Ekaza *et al* found 60% detection rate in a study with 30 patients and an attendance rate of 75% among strains [18]. There was no statistically significant difference between detection at antrum and corpus. Detection of Cag gene not depended on the sampling site (OR = 0.81).

In patients of Cag gene, a male predominance of 53.8% was noted and the most represented age group was that of over 50 years. These results are consistent with Wood Ward M *et al* and it would be probably related to greater exposure in humans [19, 20].

Unemployed accounted for 38.5% of holders of Cag gene against 15.4% for executives. The association between socioeconomic status and *H. pylori* infection is evident. Social insecurity of a population is always associated with a high prevalence in this population, probably because of overcrowding and poor hygiene that characterize this kind of population [19].

The first endoscopic indication pattern was epigastralgia pain with a rate of 38.4%. This rate is different from those found by Attia *et al.*, in Ivory Coast and Ilboudo *et al.*, Burkina Faso to 64.7% and 69.6% respectively [21, 22]. Elsewhere in Europe, Sanguino *et al*. reported epigastralgia pain in 52% of cases [23]. Also, 53.2% of patients had a family history of ulcer syndrome which brings us to the concept of family-based contamination.

Our study showed that endoscopic lesions found were not specific to the presence of the gene Cag what has been confirmed Husson MO *et al* [11]. Also among those Cag gene patients, 46.2% had a family history of ulcer syndrome. *H. pylori* is the primary cause of peptic ulcer disease and since the transmission of this organism is human to human so we can blame the family environment as the one that favored the infection in these patients.

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