

Role of *Helicobacter pylori* in the genesis of gastric ulcerations among smokers and nonsmokers

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دور الملوية البوابية في تسبب قرحات المعدة بين المدخنين وغير المدخنين
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خلاصة : تم استقصاء العدوى بالملوية البوابية بثلاث طرائق مختلفة (مصلية وجراثومية وكيميائية حيوية) بين 210 مريضا من الذكور الذين تتراوح أعمارهم بين 20 و 40 سنة . وقد شخصت قرحات المعدة في 126 مريضا كانت حالاتهم مصحوبة بعدوى الملوية البوابية بدرجة يعتد بها إحصائيا ($\chi^2 = 162.8$) . وتبين وجود معدلات عدوى عالية بين المدخنين بالمقارنة بغير المدخنين ، سواء بين المصابين بقرحات المعدة (99% مقابل 79%) أم بين غيرهم . وتم حساب خطر العدوى بهذه الجراثيم بين فئات المدخنين المختلفة . فوجدت أعلى معدلات العدوى بين من يدخنون بشراهة ، وجاء من بعدهم من يدخنون بدرجة متوسطة ثم بدرجة منخفضة وأخيرا مدخنو النرجيلة الشعبية . ولم يوجد أي خطر يعتد به بين مدخني النرجيلة الخصوصية ولا مدخني السيجار أو الغليون .

ABSTRACT *Helicobacter pylori* infection was investigated by three different methods (serologically, microbiologically and biochemically) in 210 male patients aged 20–40 years. Gastric ulcers were diagnosed in 126 patients in which *H. pylori* infection showed a significant association ($\chi^2 = 162.8$). Smokers had a higher rate of infection than nonsmokers, both among patients with gastric ulcers (99% compared to 79%) and without. The risk of *H. pylori* infection was computed for different categories of smoker. Heavy cigarette smokers were found to have the highest risk, followed by moderate to mild cigarette smokers and communal *shisha* smokers; neither private *shisha* nor cigar/pipe smokers showed any significant risk.

Rôle d'*Helicobacter pylori* dans la genèse des ulcérations gastriques chez les fumeurs et les non-fumeurs.

RESUME L'infection par *Helicobacter pylori* a fait l'objet d'investigations à l'aide de trois méthodes différentes (sérologique, microbiologique et biochimique) chez 210 patients de sexe masculin âgés de 20 à 40 ans. Des ulcères gastriques ont été diagnostiqués chez 126 patients pour lesquels une association significative avec l'infection par *H. pylori* a été établie ($\chi^2 = 162.8$). Le taux d'infection des fumeurs était supérieur à celui des non-fumeurs parmi les patients souffrant ou non d'ulcères gastriques (99% contre 79%). Le risque d'infection par *H. pylori* a été calculé chez différentes catégories de fumeurs. On a constaté que le risque le plus élevé se trouvait chez les gros fumeurs de cigarettes suivis par ceux qui fument modérément ou peu et les personnes qui fument en commun le narguilé dans les cafés et lieux publics; il n'y avait pas de risque important pour les personnes qui fument seules le narguilé à la maison ni pour celles qui fument le cigare ou la pipe.

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Introduction

The genesis of gastric ulcers has been explained by different hypotheses and factors, e.g. smoking, alcoholism, emotional stress. However, the presence of the bacillus *Helicobacter pylori*, which can be obtained by biopsy from the human antral mucosa, seems to confirm the supposition that an infectious coparticipation exists in the genesis of inflammatory lesions of the gastric and duodenal mucosa. *H. pylori* is also thought to be involved in causing metaplasia, which might progress to gastric cancer [1-4]. Recently, treatments for gastric ulceration have been reviewed. New treatments designed to attack this pathogen have been recommended in order to achieve better cure rates and lower recurrence rates [5-7].

H. pylori, formerly known as *Campylobacter pylori*, are Gram-negative microaerophilic spiral bacteria that have been identified by culture only in the past decade [8].

Different laboratory methods for diagnosis have been proposed to detect *H. pylori* infection and its disease association. The direct and indirect techniques available vary in their degree of efficiency, invasiveness and convenience. They include histological examination, culture, biochemical assay based on the characteristic metabolic activities of the organism and serological evidence of immune response [9,10].

This work aimed at studying any possible synergism between different kinds of smoking and *H. pylori* infection and association with the development of gastric ulceration.

Patients and methods

Two hundred and ten patients who needed gastroscopy (with other relevant investigations) were included in the study. Of those,

49 were nonsmokers, while the rest ($n = 161$) were smokers, categorized as follows:

- Smoker A: heavy to moderate cigarette smoker (> 20 cigarettes/day) ($n = 79$);
- Smoker B: moderate to mild cigarette smoker (≤ 20 cigarettes/day) ($n = 61$);
- Smoker C: communal *shisha* (hookah) smoker¹ ($n = 14$);
- Smoker D: private *shisha* smoker² ($n = 3$);
- Smoker E: cigar/pipe smoker ($n = 4$).

All patients were males between 20 and 40 years of age.

The patients were tested for the presence of *H. pylori* infection by three different methods.

Serologically. This was done using a newly introduced test, based on indirect solid-phase immunoassay technology (BioSign *H. pylori* WB-PBM, New Jersey, USA). In the test procedure, 25 ml of whole blood or 10 ml of serum is spotted in the sample well. If any anti-*H. pylori* antibody is present in the sample, it will be captured by the *H. pylori* antigen band impregnated in the test membrane. The specimen followed by the developer solution moves by capillary action to the antigen band, the solution mobilizes the dye conjugated to anti-human Ig antibodies. Visualization of the antigen band in the test window occurs only when the antibody-dye conjugate binds to the anti-*H. pylori* antibodies (IgA/IgG/IgM) which have been bound to *H. pylori* antigen.

Microscopically (Gram stain). Tissue sample from the prepyloric antrum taken dur-

¹Shisha used communally in coffee shops and other public places.

²Shisha used at home by one person.

ing gastroscopy was smeared using a sterile loop by rubbing it forcefully over a sterile glass microscopic slide (touch technique) [11]. All slides were labelled, air dried, heat fixed and Gram stained using dilute carbol fuchsin (0.3%) as a counter stain. A Gram-negative either spiral or curved bacillus or the classic gull-wing appearance was interpreted as *H. pylori* [12].

Biochemically (urease). This was done using the Jatrox-H.p test from Röhm Pharma GmbH, Weiterstadt, Germany [13]. The principle of this test is that the urea present in the test medium is split by the urease present in the *H. pylori*. The rise in the pH value associated with the splitting of the urea causes a colour change in the indicator (phenol red), which is also present in the test medium, from yellow to pink/red. The same biopsy specimen which was used for the Gram stain touch preparation was introduced into the test medium (one tablet of urea 97.720% and phenol red 0.017% dissolved in 0.5 ml distilled water). Reaction was observed within 30 minutes. If the test result was negative (no colour change), further checks were made after 3 hours and 24 hours (for low levels of *H. pylori*); any colour changes after that were not considered.

Results

In this study, only those patients who showed positive results for all tests applied (serology, Gram stain and urease) were considered positive for *H. pylori* infection and were included in the statistical analysis.

H. pylori infection was detected by all three tests in 128 out of the total of 210 cases examined (61%). The rate was higher if each test was taken individually (78% with serology, 62% with Gram stain and 66%

with urease test). Infection prevailed mostly (70%) in smokers (113 out of 161). According to odds ratio, the risk of infection was 5.3 times higher for smokers than non-smokers, which was significant (Table 1). The risk of infection among private *shisha* or cigar/pipe smokers was statistically not significant (1.1 and 0.8 respectively); heavy cigarette smokers showed the highest significant risk (8.2) followed by moderate to mild cigarette smokers and communal *shisha* smokers (4.3 and 4.1 respectively).

Gastroscopy confirmed the presence of gastric ulcerations in 126 patients (60%), while it was negative for the other 84 patients. *H. pylori* infection was found in 96% of patients with gastric ulcers, whereas it was found in only 8% of patients without ulceration, making infection with ulceration statistically significant ($\chi^2 = 162.8$) (Table 2).

It was also noticed that smokers were statistically more infected with *H. pylori* (99%) than nonsmokers (79%) in patients with gastric ulcers (Table 3).

Discussion

In this study, *H. pylori* infection was diagnosed by the three tests applied in 61% of 210 patients subjected to upper gastrointestinal endoscopic examination. This rate is similar to those reported by other investigators such as Buck et al. [14] in United States of America, who detected the organism in 69% of 39 patients with gastritis. Higher rates of infection were reported in other studies, e.g. 77% in the Netherlands by Rauws et al. [15] and 79.4% in Kuwait by Britt et al [16]. However, these higher rates could be reached or even exceeded when we compare infection rates of smokers (up to 78% as shown in Table 1) or pa

Table 1 Positive test(s) for *H. pylori* infection among different smokers and nonsmokers

Group of patients ^a	Serology		Gram stain		Urease		Positive for all tests		Risk ratio	
	No.	%	No.	%	No.	%	No.	%	OR	95% CI
Smoker A (n = 79) significant	72	91	63	80	65	82	62	78	8.2	3.4–20.3
Smoker B (n = 61) significant	54	89	40	66	42	69	40	66	4.3	1.8–10.5
Smoker C (n = 14) significant	12	86	9	64	10	71	9	64	4.1	1.01–17.24
Smoker D (n = 3) not significant	2	67	1	33	1	33	1	33	1.1	0.04–18.11
Smoker E (n = 4) not significant	2	50	1	25	1	25	1	25	0.8	0.03–9.45
Total smokers (n = 161) significant	142	88	114	71	119	74	113	70	5.3	2.53–11.4
Nonsmokers (n = 49)	22	45	17	35	20	41	15	31		
Total (n = 210)	164	78	131	62	139	66	128	61		

^aSmoker A: heavy to moderate cigarette smoker; Smoker B: moderate to mild cigarette smoker; Smoker C: communal shisha smoker; Smoker D: private shisha smoker; Smoker E: cigar/pipe smoker
OR = odds ratio; CI = confidence interval

Table 2 *H. pylori* infection according to presence of gastric ulceration

Infection	With ulcer		Without ulcer		Total	
	No.	%	No.	%	No.	%
Positive	121	96	7	8	128	61
Negative	5	4	77	92	82	39
Total	126	100	84	100	210	100

$\chi^2 = 162.8$; $P < 0.05$ (significant)

tients with confirmed gastric ulcers (96% as shown in Table 2). Increased infection has been observed with age [17,18] but the ages of our patients ranged only between 20 and 40 years, which might explain our relatively low infection rate.

Table 3 *H. pylori* infection among smokers and nonsmokers with gastric ulceration

Infection	Smokers		Nonsmokers		Total	
	No.	%	No.	%	No.	%
Positive	106	99	15	79	121	96
Negative	1	1	4	21	5	4
Total	107	100	19	100	126	100

Two-tailed Fisher exact $P = 0.00005$ (significant)

Generally, smokers were found to have a statistically significant higher risk (risk ratio = 5.3) of *H. pylori* infection than nonsmokers (Table 1). This finding is in agreement with Martin et al. [19], who reported there was a relationship between *H. pylori*

infection and smoking, but not with Chodos et al. [20], who reported no significant relationship.

Cigarette smoking was found to have the highest statistically significant correlation with *H. pylori* infection. This was more pronounced with heavy to moderate than moderate to mild cigarette smoking. A significant correlation was also found with communal *shisha* smoking, while no significant risk of *H. pylori* infection was found in either private *shisha* or cigar/pipe smokers (Table 1). These results could be interpreted in different ways. Smoking has a destructive effect on the immunity of gastric mucosa and lining layers and hence increases its susceptibility to infection by *H. pylori*. Communal *shisha* smoking might carry the risk of passing the infection from

a diseased person to an uninfected one, as oral-to-oral infection has been documented by many authors [21,22]; on the other hand, this risk would be virtually absent in private *shisha* smoking. The insignificant correlation between *H. pylori* infection and cigar/pipe smoking might be explained by the two following observations: the low frequency of smoking in this group and the relatively higher socioeconomic standard observed in this group of smokers. The latter observation is consistent with other studies [23,24], which demonstrated that patients of a high socioeconomic standard suffering from upper gastrointestinal symptoms had significantly lower rates of infection. This emphasizes the importance of socioeconomic standard as a risk factor in *H. pylori* infection.

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