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Clinical Trial

Helicobacter pylori cagA Gene in Egyptian Sewage Workers

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Abstract A comparative cross-sectional study was conducted on 60 sewage workers and 30 matched unexposed referents from Mansoura city, Egypt, to estimate the prevalence and risk factors of *Helicobacter pylori* (*H. pylori*). Stool culture and detection of *H. pylori* antigen were done. In addition, the polymerase chain reaction (PCR) amplification of cytotoxin-associated gene A (cagA) gene in *H. pylori* in stool samples was carried out. The prevalence of *H. pylori* in sewage workers was 56.7% compared to 16.7% in the comparison group with a highly statistically significant difference between both groups. *H. pylori* cagA gene was present in 64.7% of *H. pylori*-infected sewage workers compared with 40% of controls. cagA gene was associated with more dyspeptic symptoms (77.3%) in infected workers compared to those of noninfected ones (33.3%) ($P = .041$). The risk of *H. pylori* was significantly higher among workers with poor compliance with personal protective equipment (PPE) (OR = 3.00), with duration of work > 20 years (OR = 4.71), older than 45 years (OR = 4.27), and of low education level (OR = 11.2). We concluded that *H. pylori* infection and heartburn with or without epigastric pain are significant health problems in the studied sewage workers. Low education and poor compliance with PPE were the only predictors of *H. pylori* infection in sewage workers.

Keywords *H. pylori*; gastrointestinal symptoms; sewage workers

1. Introduction

Helicobacter pylori (*H. pylori*) are one of the most common chronic infections worldwide [14]. It affects approximately 50% of the world population and is more prevalent in developing countries [19]. It causes gastritis and peptic ulceration. It is an important risk factor for gastric adenocarcinoma and the second highest cause of cancer deaths worldwide [17]. However, the majority of infected subjects develop no clinical symptoms [22].

In Egypt, prevalence of *H. pylori* infection was reported to be 60% up to 86% of adult population [8]. This prevalence is considered a public health problem especially among low socioeconomic-level populations, probably due to the conditions that favor the acquisition of infection such as precarious hygiene, crowded living conditions, absence or deficiency of sanitation, and poor hygiene [10].

The cytotoxin-associated gene A (cagA) gene is associated with type I strains which are responsible for severe forms of gastrointestinal diseases. It is present in approximately 50% to 70% of *H. pylori* strains [24].

The occupational risk for sewage-exposed workers for developing *H. pylori* infections has received a little attention and no data were available on the validation of noninvasive tests for the diagnosis of *H. pylori* among sewage workers in Egypt.

Our study aimed to estimate the prevalence of *H. pylori* infections, to genotype the isolated strains among this vulnerable group of workers, and to assess the risk factors for developing *H. pylori* infection.

2. Subjects and methods

2.1. Study design

A comparative cross-sectional study was conducted during the period from 1st January to 31 August 2010. The exposed group included 60 sewage workers in Mansoura sewage treatment plant and maintenance of sewage collection system. The control group consisted of 30 food workers in Mansoura University Hospital. The study was approved by the Research Ethics Committee of Mansoura University. Both groups gave a written informed consent to participate in the study.

2.2. Study tools

All recruited subjects were subjected to the following procedures.

(1) Compliance with the personal protective equipment (PPE) as overall, plastic boots, plastic gloves, and masks was assessed using a scoring system depending on time of wearing: most of the time was given a score (2), part of the time was given a score (1), and never was given a score (0). Poor compliance was considered when the total score was less than (4).
(2) Stool and EDTA blood samples were given for laboratory assessment.

Table 1: Sociodemographic criteria of the sewage workers compared to unexposed referents.

Sociodemographic criteria	Sewage workers ($n = 60$)	Referents ($n = 30$)	Test of significance	P-value
Age (y)				
Mean ±SD	37.68 ± 10.81	37.80 ± 6.34	$t = 0.054$	0.95
24–35	31 (51.7%)	10 (33.3%)		
36–45	14 (23.3%)	13 (43.3%)	$\chi^2 = 4.16$	0.125
> 45	15 (25%)	7 (23.3%)		
Smoking				
Nonsmokers	42 (70%)	23 (76.7%)	$\chi^2 = 0.443$	0.621
Smokers	18 (30%)	7 (23.3%)		
Residence				
Rural	35 (58.3%)	21 (70%)		
Suburban	7 (11.7%)	3 (10%)	$\chi^2 = 1.238$	0.53
Urban	18 (30%)	6 (20%)		
Marital status				
Married	52 (86.7%)	26 (86.7%)	$\chi^2 = 0.11$	0.74
Single	8 (13.3%)	4 (13.3%)		
Education				
$<$ secondary	43 (71.7%)	15 (50%)		
Secondary	10 (16.7%)	7 (33.33%)	$\chi^2 = 7.643$	0.054
$>$ secondary	7 (11.7%)	8 (26.66%)		
Duration of employment (y)				
Mean ±SD	12.37 ± 11.15	15.36 ± 6.20	$t = 1.365$	0.17
< 10	32 (53.3%)	9 (30%)		
10–20	14 (23.3%)	14 (46.7%)	$\chi^2 = 5.24$	0.072
> 20	14 (23.3%)	7 (23.3%)		

2.3. Laboratory procedures

(1) *Complete blood count:* using Sysmex.

(2) *Stool analysis:* using the spontaneous sedimentation method.

(3) *Stool culture.*

(4) *Detection of H. pylori antigen in stool:* using an immunochromatographic rapid assay (Cal-Tech Diagnostics, Inc., Chino, CA, USA). *H. pylori* antigen band test is a sandwich solid-phase immunochromatographic assay.

(5) *H. pylori PCR for cagA gene [18]:* stool samples which were selected for PCR amplification of cagA gene were positive for *H. pylori* antigen. Only 34 specimens fulfilled this criterion and were candidates for amplification by PCR, illustrated in the following:

- DNA extraction: using the QIAamp DNA stool minikit (Qiagen) according to the manufacturer's instructions.
- PCR: a 349-bp target sequence from cagA gene was amplified using two primers: forward primer P1: 5′-GATAACAGGCAAGCTTTTGAGG-3′ and reverse primer P2: 5′-CTGCAAAAGATTGTTTGCGAGA-3′.

The PCR was performed in a total volume of 50 μL of master mix (EzWay PCR Master Mix, Koma Biotech, Seoul, Korea) containing 1 μL of the extracted DNA and 0.5 μM of each primer.

2.4. Statistical analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 11 (SPSS Inc., Chicago, IL, USA). The study groups were compared regarding demographic criteria, gastrointestinal symptoms, and laboratory data using the Chi-square test and Student's t-test. Significant factors predicting *H. pylori* infection on bivariate analysis were entered into multivariate logistic regression analysis to find out the independent predictors of *H. pylori* infection. The odds ratio and 95% confidence interval were calculated. $P \leq .05$ was considered statistically significant.

3. Results

Table 1 summarizes the demographic data of the studied groups. As shown in Table 2, heartburn with or without epigastric pain symptoms was significantly higher in sewage workers (43.3%) compared with that in the unexposed referents (20%) ($P \leq .05$).

Table 3 demonstrated that the prevalence of *H. pylori* in sewage workers was 56.7% and that there was a highly statistically significant difference compared to unexposed referents ($P \leq .001$). Most of both groups were positive for cagA gene (64.7% and 40%) without a significant difference.

By univariate analysis, the risk of *H. pylori* was significantly higher among workers with poor compliance with PPE (OR = 3.00, 95% CI: 1.07–10.35), workers with duration of work > 20 years (OR = 4.71, 95% CI: 1.10-20.20), workers older than 45 years (OR = 4.27, 95% CI: 1.00–18.15), and low-educated (less than secondary) workers (OR = 11.2, 95% CI: 1.23–101.89) (Table 4).

Table 2: Frequency of gastrointestinal symptoms in sewage workers compared to unexposed referents.

Gastrointestinal symptoms	Sewage workers ($n = 60$)	Referents ($n = 30$)	Test of significance	P-value
Heartburn ± epigastric pain	26 (43.3%)	6 (20%)	$\chi^2 = 3.79$	0.05*
Colic	10 (16.7%)	10 (33.33%)	$\chi^2 = 3.21$	0.07
Changes in bowel habit	3 (5%)	3 (10%)	$\chi^2 = 0.80$	0.396
Distension	8 (13.3%)	2 (6.7%)	$\chi^2 = 0.9$	0.486

*Statistically significant ($P \leq .05$).

Table 3: Comparison between sewage workers and unexposed referents regarding stool and hematological parameters.

Stool and hematological parameters	Sewage workers ($n = 60$)	Referents ($n = 30$)	Test of significance	P-value
***Helicobacter pylori* Ag**	34 (56.7%)	5 (16.7%)	$\chi^2 = 13.03$	0.000**
cagA gene	22/34 (64.7%)	2/5 (40%)	$\chi^2 = 0.323$	0.57
Stool examination				
E. histolytica	39 (65%)	17 (56.6%)	$\chi^2 = 0.590$	0.442
G. lamblia	12 (20%)	3 (10%)	$\chi^2 = 1.44$	0.230
Stool culture[a]				
Proteus	23 (38.3%)	14 (46.7%)	$\chi^2 = 0.28$	0.59
Klebsiella	28 (46.7%)	8 (26.7%)	$\chi^2 = 3.33$	0.07
E. coli	9 (15%)	8 (26.7%)	$\chi^2 = 1.78$	0.18
Hb	12.52 ± 1.04	12.10 ± 1.55	$t = 1.53$	0.130
RBCs	4.37 ± 0.30	4.26 ± 0.54	$t = 1.18$	0.242
Ht	38.16 ± 3.44	38.05 ± 5.17	$t = 0.12$	0.903
WBCs	5.75 ± 2.39	6.55 ± 2.80	$t = 1.42$	0.158
Platelets	183.65 ± 37.35	214.76 ± 49.3	$t = 2.56$	0.012*

[a]No pathogenic organisms were detected in both groups.
*Statistically significant ($P \leq .05$).
**Highly statistically significant ($P \leq .001$).

Table 4: Risk factors of *H. pylori* infection among sewage workers.

	Positive *H. pylori* ($n = 34$)	Negative *H. pylori* ($n = 26$)	OR (95% CI)	P-value
PPE adequate compliance	17 (50%)	20 (76.93%)	r	—
PPE poor compliance	17 (50%)	6 (23.07%)	3.00 (1.07–10.35)	0.03*
Nonsmokers	21 (61.8%)	21 (80.8%)	r	—
Smokers	13 (38.2%)	5 (19.2%)	2.60 (0.78–8.59)	0.15
Duration of work (years)				
< 10	14 (41.2%)	18 (69.2%)	r	—
10–20	9 (26.5%)	5 (19.2%)	2.31 (0.63-8.47)	0.19
> 20	11 (32.4%)	3 (11.5%)	4.71 (1.10-20.20)	0.02*
Age (years)				
24–35	15 (44.1%)	16 (61.5%)	r	—
36–45	7 (20.6%)	7 (26.9%)	1.07 (0.3–3.77)	0.92
> 45	12 (35.3%)	3 (11.5%)	4.27 (1.00–18.15)	0.05*
Residence				
Rural	17 (50%)	18 (69.2%)	r	—
Suburban	4 (11.8%)	3 (11.5%)	1.41 (0.27–7.26)	0.67
Urban	13 (38.2%)	5 (19.2%)	2.75 (0.81–9.38)	0.10
Education				
$<$ secondary	28 (82.35%)	15 (57.69%)	11.2 (1.23–101.89)	0.03*
Secondary	5 (14.70%)	5 (19.23%)	6.0 (0.52–69.76)	0.31
$>$ secondary	1 (2.94%)	6 (23.07%)	r	—

OR: odds ratio, CI: confidence interval, r: reference group.
*Statistically significant ($P \leq .05$).
PPE: personal protective equipment.

Table 5: Logistic regression analysis of independent predictors of *H. pylori* infection among sewage workers.

Predictors	B	P	OR (95% CI)
PPE compliance			
Adequate compliance	—	—	r
Poor compliance	1.65	0.01*	5.21 (1.49–18.20)
Education			
$<$ secondary	3.76	0.008*	43.35 (2.61–717.99)
Secondary	1.25	0.19	3.49 (0.52–23.25)
$>$ secondary	—	—	r
Constant	−1.76	0.07	—

OR: odds ratio, CI: confidence interval, r: reference group.
*Statistically significant ($P \leq .05$).
PPE: personal protective equipment.

The independent predictors of *H. pylori* infection among sewage workers as revealed by logistic regression analysis are presented in Table 5. Low education and poor compliance with PPE (OR = 43.35 and 5.21, resp.) were the only significant predictors of *H. pylori* infection among sewage workers.

A highly statistically significant association between heartburn with or without epigastric pain and infection with *H. pylori* ($P \leq .001$) was detected in the exposed group. On the other hand, there were no statistically significant associations between hematological parameters such as anemia, thrombocytopenia, and infection with *H. pylori* (Table 6).

As shown in Table 7, *H. pylori* cagA positive sewage workers were complaining more frequently from heartburn with or without epigastric pain than the nonvirulent infected workers ($P \leq .05$).

4. Discussion

Nearly, for the first time, the current study shows a higher seroprevalence of *H. pylori* infection in Egyptian sewage workers when compared with control participants, matched for age, sex, and socioeconomic status.

The sensitivity of stool cultures compared to tissue-based assays and stool antigen testing was 21% while the specificity was 100% [13]. Detection of *H. pylori* by PCR from nonbiopsy samples has been widely used recently in many laboratories because it is a noninvasive method and because of its rapid nature, especially when results are needed for emergency cases [2].

Comparable to *H. pylori* PCR, the stool antigen assays showed higher sensitivities and lower specificities than those of PCR but well comparable to those of breath tests [4] as follows: the sensitivity and specificity of the monoclonal stool antigen test were 97% and 94%, respectively [6], the sensitivity and specificity of HpSA test and stool PCR were 80% and 83.3% and 72.5% and 100%, respectively [15], and Blanco et al. [3] reported that *H. pylori* ICT methods showed a higher sensitivity and specificity (92.1% and 100%).

In our study, we used one of these IC kits to assess *H. pylori* infection in sewage workers with detection rate to be 56.7% compared to 16.7% among unexposed referents with highly statistically significant difference between both groups. This was to some extent in accordance with the findings of Jeggli et al. [12] who detected the prevalence of IgG Ab to *H. pylori* of around 43%; compared to Van Hooste et al. [26] who observed a lower prevalence than our study of around 16.7% compared to 13.6% among the control group. In some poor locality in Northeastern Brazil, the prevalence of *H. pylori* in two studies was very high (80% and 62.9%) [20, 21]. This much variation in the prevalence of *H. pylori* may be due to locality or environmental variation or due to the difference in the method of the detection of *H. pylori* as well as poor control measures for exposure to raw sewage particles.

The cagA gene has been described as a marker for pathogenic *H. pylori* strains and many studies suggested a correlation between the prevalence of cagA seropositivity and peptic ulcer disease and gastric cancer [1]. Our results showed that cagA gene *H. pylori* was positive in 64.7% of sewage workers and 40% of control referents. Most of them (77.3%) were complaining of heartburn and epigastric pain. Similarly, it is known before that the cagA *H. pylori* gene was detected in 42% of patients who were endoscopically diagnosed as normal. The most likely explanation is that the type IV secretion apparatus in the pathogenic *H. pylori* isolated from these patients might be switched off or defective [16]. Another explanation is that the immune system of these individuals might be defective or the general immunity is low for some reason. It has been noted before that these immune system cells have an important role in gastric inflammation [7].

Prevalence of cagA gene in Egypt was 35.7% in a study which has done by Hussein in the Middle East [11]. Another study from Egypt reported the findings from 28 isolates most of which had been obtained from patients with nonulcer dyspepsia. The prevalence of cagA was also low (e.g., 36%) [25]. The prevalence of cagA in Egypt was related to the clinical presentation of *H. pylori* infection: being the lowest in asymptomatic controls (11.1%) and increasingly prevalent in nonulcer dyspepsia (40%), peptic ulcer (66.7%), and gastric cancer (89%) [24]. The prevalence of cagA positive *H. pylori* strains varies from one geographic region to another, for example, 72% in Germany, 67% in the Netherlands, 48% in Sri Lanka, 81% in the United States, 93% in Nigeria, and 97% in Korea [23].

Heartburn with or without epigastric pain was significantly higher in our sewage workers group (43.3%) as compared with the unexposed referents. This was in accordance with the findings of Friis et al. [9] and Van Hooste et al. [26] who found that the sewage workers were affected more often by peptic ulcers during their

Table 6: Gastrointestinal symptoms and hematological abnormalities in *H. pylori* positive and negative sewage workers.

	Positive *H. pylori* ($n = 34$)	**Negative *H. pylori*** ($n = 26$)	**Test of significance**	P**-value**
Heartburn and/or epigastric pain	21 (61.8%)	5 (19.2%)	$\chi^2 = 10.85$	0.001**
Colic	7 (20.6%)	3 (11.5%)	$\chi^2 = 0.869$	0.49
Changes in bowel habits	1 (2.9%)	2 (7.7%)	$\chi^2 = 0.70$	0.57
Distension	5 (14.7%)	3 (11.5%)	$\chi^2 = 0.12$	1.00
Anemia	22 (64.7%)	12 (46.2%)	$\chi^2 = 2.06$	0.19
Thrombocytopenia	2 (5.9%)	4 (15.4%)	$\chi^2 = 1.47$	0.38

**Highly statistically significant ($P \leq .001$).

Table 7: Gastrointestinal symptoms in cagA *H. pylori* positive and negative sewage workers.

	Positive cagA *H. pylori* ($n = 22$)	**Negative cagA *H. pylori*** ($n = 12$)	**Test of significance**	P**-value**
Heartburn and/or epigastric pain	17 (77.3%)	4 (33.3%)	$\chi^2 = 4.62$	0.031*
Colic	3 (14.3%)	4 (30.8%)	$\chi^2 = 0.835$	0.361
Changes in bowel habits	1 (4.8%)	0 (0%)	—	—
Distension	2 (9.5%)	3 (23.1%)	$\chi^2 = 0.555$	0.456

*Statistically significant ($P \leq .05$).

present jobs than the referents. On the other hand, the other gastrointestinal symptoms showed no significant difference between sewage workers and unexposed referents. This also was in agreement with the study conducted by Friis et al. [9].

In the present study, the highest prevalence of *H. pylori* was shown in sewage workers who live in rural area and of low education level. This was in agreement with the study of Rodrigues et al. [21] which was on *H. pylori* infection in adults from a poor community in Northeastern Brazil. The risk of *H. pylori* was significantly higher among workers with low education, poor compliance with PPE, duration of work > 20 years, and age > 45 years. This was in agreement with the observations of Van Hooste et al. [26] who reported a high risk of *H. pylori* seropositive status with longer duration of work. On logistic regression, low education and poor compliance with PPE were the only significant predictors of infections. An increasing body of published scientific evidence suggests that exposure to environmental and occupational hazards is not randomly distributed in the population, but tends to be disproportionately concentrated among communities of people of low education and socioeconomic level, as well as among low-income workers and workers of low education. This could be explained by unawareness of the health hazards, neglecting protective measures, and inadequate healthcare [5].

From this study, we concluded that *H. pylori* infection and heartburn with or without epigastric pain are significant health problems in the studied sewage workers. Low education and poor compliance with PPE were the only significant predictors for *H. pylori* infection in sewage workers.

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