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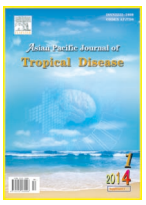

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Detection of *Helicobacter pylori* infection in patients with obstructive airway diseases with sero techniques using highly specific IgG antibodies for *Helicobacter pylori* antigen

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PEER REVIEW

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Comments

This is a good study in which authors evaluated the correlation between *H. pylori* infection and obstructive airway diseases (COPD and asthma). This relationship can be confirmed only through IIFA investigations. In this regard this study is the first of its kind and no reports are available for comparison purpose.
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ABSTRACT

Objective: To determine the authenticity of three techniques *viz.*, ELISA, western blot and indirect immune fluorescence assay (IIFA) to establish the connection between *Helicobacter pylori* (*H. pylori*) and two obstructive airway diseases, chronic obstructive pulmonary diseases (COPD) and asthma.
Methods: Serum samples were collected from 48 patients, 27 with COPD, 21 with asthma diseases and control sera were obtained from 42 healthy volunteer blood donors. Serum samples were analyzed by three sero-based techniques, *viz.*, ELISA, western blot and IIFA.
Results: ELISA results revealed no connection between the *H. pylori* infection and COPD and asthma. Western blot results also did not reveal any relationship between *H. pylori* and obstructive airway diseases. Antibody pattern also did not support the connection between these two diseases. IIFA tests revealed a positive connectivity and relation between the two diseases.
Conclusions: The results of the present investigations reveal an association of *H. pylori* in COPD and asthma. IIFA is a reliable test and hence it is recommended.

KEYWORDS

Helicobacter pylori, Asthma, Chronic obstructive pulmonary diseases, ELISA, Western blot, Indirect immune fluorescence assay

1. Introduction

Helicobacter pylori (*H. pylori*) infection affects an approximately 50% of the world population[1]. It is well known that this bacterium possesses a well defined battery of virulence factors, which allow the organism to colonize the gastric mucosa, evade host defence and finally damage host tissue[2]. Extensive clinical trials carried out in the past few years have proved the role of *H. pylori* as the main cause of both chronic gastrititis and peptic ulcer disease[3,4]. This bacterium is also casually related to low grade B-cell lymphoma of gastric mucosa associated lymphoid tissue[5]. Moreover, *H. pylori* infection has been established as a risk factor for the development of gastric adenocarcinoma[6]. Finally, recent studies indicate that *H. pylori* might be related to non ulcer dyspepsia[7]. Recent

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studies suggest an increased *H. pylori* prevalence in patients with various extra gastrointestinal disorders, including skin, cardiovascular, rheumatic and liver diseases[8-10]. Incidence of some respiratory diseases was linked to a high prevalence of *H. pylori* infection[11,12]. The observed association may be explained due to a potential etiopathogenic role of *H. pylori* infection in extra digestive disorders[13]. However, at present, there is no definite proof of a casual relationship between *H. pylori*, the two obstructive airway diseases, chronic obstructive pulmonary disease (COPD) and asthma diseases[14]. In view of suspected and unconfirmed association of *H. pylori* in COPD and asthma, in the present investigations an attempt was made to establish the link, if any, between these diseases through sero-techniques like ELISA, western blot and indirect immunofluorescence assay (IIFA).

2. Materials and methods

2.1. Patients

The present investigations were carried out at the A.P State Chest Diseases and Tuberculosis Centre, Hyderabad, India. Consecutive patients with diagnosed and confirmed COPD and asthma diseases attending the center were selected for the present study. The prospective study had the Institutional Ethical Committee approval. A written consent was obtained from the selected patients. Control group included healthy subjects, well matched for age, sex, nutritional status and socioeconomic status from a health camp conducted specifically for this purpose. None of the control subjects had a known history of COPD, asthma, upper gastrointestinal tract pathology and any clinical manifested diseases. The subjects included in this study were 27 COPD patients (17 males, 10 females), 21 asthma patients (10 males, and 11 females) and 42 control (21 males and 21 females). A questionary included the demographic characteristics like gender, age, socio-economic status, profession *etc*. Five milliliter of blood was collected from both diseased and healthy subjects for serological tests.

2.2. Serological tests

2.2.1. ELISA

Seropositivity of *H. pylori* infection in COPD and asthma subjects and control group was tested with commercially available anti-*H. pylori* IgG (EUROIMMUN Mediznische Labordiagnostika, Lubeck, Germany) as per the instructions of the manufacturers. The optical density of the resultant colour was calculated from the extinction value of the subjects sample over the extinction value of the calibrator 2. The ELISA recommended values>1.1 (U/mL) were taken as positive; between 0.8-1.1 (U/mL) are treated as borderline positive and <0.89 (U/mL) were treated as negative[15].

2.2.2. Western blot

Western blot was performed to detect the seropositivity of *H. pylori* infection in COPD, asthma and control subjects. Anti-*H. pylori* IgG antibodies in the serum were detected using commercially available western blot strips (EUROIMMUN Mediznische Labordiagnostika, Lubeck, Germany) according to the manufacturer's instructions. Western blot test strips consisted of antigen extracts with the following molecular weights:120 kDa (cagA); 95 kDa (vacA); 67 kDa (flagellar sheath protein, nonspecific); 66 kDa (ureB); 57 kDa (heat-shock protein homolog); 33 kDa, 30 kDa, 29 kDa (ureA); 26 kDa, 19 kDa and 17 kDa. However, Anti-*H. pylori* IgG antibodies positivity was determined when the 120 kDa (cagA) band, as well as at least two distinctive antigen bands from species-specific and highly specific antigens with the molecular weights of 95 kDa (vacA), 33 kDa, 30 kDa,29 kDa (ureA), 26 kDa, 19 kDa and 17 kDa were present. Faint bands or no band were treated as negative[16].

2.2.3. IIFA

Indirect immunofluorescence assay was conducted with Euroimmune Biochip slides coated with *H. pylori* bacterial smear tagged with fluorescentlabelled anti-human IgG (goat) which is designed exclusively for the *in vitro* determination of human antibodies in serum. The specific antibodies are labeled with a compound that makes them glow in an apple green color when observed microscopically under ultraviolet light. If the sample is positive, specific antibodies in the diluted serum sample attach to the antigens coupled to a solid phase and thus a distinct fluorescence of the bacteria covering the reaction areas becomes visible. The results are correlated with both positive and negative controls. Depending on the sample, the fluorescence pattern appears in parts circular or granular. In the case of a negative result, the cells show no fluorescence[17,18].

2.3. Statistical analysis

Results are expressed as mean and one standard deviation (SD). Significance of difference between groups was assessed by unpaired students *t* tests for continuous variables and *Chi*-square test for proportions. Correlation coefficients between variables were determined using conventional Pearsons correlation analysis. The statistical analysis was performed using the SPSS program (SPSS, Inc, IL, USA). *P* values less than 0.005 were considered statistically significant. Control and patients with 100% positive results are not applicable for statistical analysis.

3. Results

The results pertaining to *H. pylori* association with COPD and asthma as revealed by ELISA tests are presented in Table 1. It is evident from the critical study of the table that out

of 27 subjects suffering from COPD, 24 (88.9%) were proved to be *H. pylori* positive, whereas out of 42 control subjects 35 were proved to be positive (83.3%). The results indicated that no significant correlation ($P<0.522$) could be drawn with regard to association of *H. pylori* infection in patients suffering from COPD. Demographic-wise analysis results revealed that among male, out of 17, 14 (82.4%) patients have shown seropositivity, whereas in females there was 100% seropositivity for *H. pylori*. However, when compared with controls the correlation does not appear to be significant ($P<0.912$ and $P<0.209$). COPD patients within the age group of 20-40 years have shown 100% seropositivity with ELISA. However, in comparison with control there is no significant correlation ($P<0.249$). Overweight COPD patients have shown 100% seropositivity for *H. pylori* infection. Similarly low income and high income groups people have also shown 100% seropositivity and thus in all the above cases there is no significant correlation.

Table 1

Demographic-wise *H. pylori* association in obstractive airway diseases and control subjects through ELISA test.

Demographic		COPD vs. control	Significance value	Asthma vs. control	Significance value
Gender	Male	14/17 (82.4%)		9/10 (90.0%)	
		17/21 (81.0%)	$P<0.912$	17/21 (81.0%)	$P<0.522$
	Female	10/10 (100.0%)		7/11 (63.6%)	
		18/21 (85.7%)	$P<0.209$	18/21 (85.7%)	$P<0.151$
	Total	24/27 (88.9%)		16/21 (76.2%)	
		35/42 (83.3%)	$P<0.522$	35/42 (83.3%)	$P<0.496$
Age group	20-40	11/11 (100.0%)		8/10 (80.0%)	
		24/27 (88.9%)	$P<0.249$	24/27 (88.9%)	$P<0.482$
	41-60	8/10 (80.0%)		7/10 (70.0%)	
		11/15 (73.3%)	$P<0.702$	11/15 (73.3%)	$P<0.856$
	Above 61	5/6 (83.3%)		1/1 (100.0%)	
		0/0 (0.0%)	NA	0/0 (0.0%)	NA
Nutritional Status (BMI)	Normal	9/10 (90.0%)		1/14 (78.6%)	
		12/14 (85.7%)	$P<0.754$	12/14 (85.7%)	$P<0.429$
	Overweight	2/2 (100.0%)		0/0 (0.0%)	
		2/3 (66.6%)	$P<0.361$	2/3 (66.6%)	$P<0.277$
	Underweight	13/15 (86.7%)		5/7 (71.4%)	
		21/25 (84.0%)	$P<0.819$	21/25 (84.0%)	$P<0.077$
Socioeconomic Status	Low income	9/9 (100.0%)		8/9 (88.9.%)	
		15/17 (88.2%)	$P<0.284$	15/17 (88.2%)	$P<0.960$
	Middle income	7/10 (70.0%)		6/7 (85.7%)	
		11/13 (84.6%)	$P<0.400$	11/13 (84.6%)	$P<0.948$
	High income	8/8 (100.0%)		2/5 (40.0%)	
		9/12 (75.0%)	$P<0.125$	9/12 (75.0%)	$P<0.169$

Statistical test shows that less than 0.005 *P*-value is significant, more than 0.005 *P*-value is not significant and statistical test not applicable (NA).

In case of asthma, out of 21 patients, 16 (76.2%) proved to be ELISA positive and similarly out of 42 control subjects 35 (83.3%) were proved to be ELISA positive. Even in this case also there is no significant correlation ($P<0.496$). Male patients have shown more (90%) seropositivity than female patients (63.6%). But in comparison to control subjects no such significance was observed. Age group wise analysis also revealed that there was little variation [20-40 years (88.9%), 41-60 years (70.0%) and above 61 years (100.0%)]. However, when compared to controls seropositivity, no significant correlation could be drawn. From nutritional status point of view also no statistical significant values were obtained. The low and middle income group asthma patients have revealed more seropositivity (88.9% and 85.7%) than the high income group patients. Nevertheless, no definite correlation could be drawn between the relationship of COPD and *H. pylori* infection.

The results pertaining to investigations on *H. pylori* association with COPD and asthma as revealed by western blot are presented in Table 2. A critical perusal of the table revealed that all the 21 subjects suffering from COPD have proved to be *H. pylori* positive (100%). Similarly, all the 20 control subjects were proved to be positive (100%). Thus, there was no significance between the association of *H. pylori* infection and COPD. Demographic-wise analysis of western blot results revealed that gender wise, out of 11 male patients with COPD, 11 (100%) and out of 10 female all the 10 (100%) have proved to be positive for *H. pylori*, whereas out of 10 male control subjects 10 (100%) and out of 10 female 10 (100%) have shown positivity for *H. pylori*. Statistically, the relation between *H. pylori* infection and COPD was not significant both in males and females. With regard to different age groups, there was no significant relation between *H. pylori* infection and COPD. Among normal, overweight and underweight populations, statistics does not show any significance. Similarly socioeconomic status -wise results also did not reveal any relation between infection and COPD.

Table 2

Demographic-wise seropositivity among the obstractive airway diseases to control by Western blot.

Demographic		COPD vs. control	Significance value	Asthma vs. control	Significance value
Gender	Male	11/11 (100.0%)		10/10 (100.0%)	
		10/10 (100.0%)	NA	10/10 (100.0%)	NA
	Female	10/10 (100.0%)		10/10 (100.0%)	
		10/10 (100.0%)	NA	10/10 (100.0%)	NA
	Total	21/21 (100.0%)		20/20 (100.0%)	
		20/20 (100.0%)	NA	20/20 (100.0%)	NA
Age group	20-40	10/10 (100.0%)		10/10 (100.0%)	
		13/13 (100.0%)	NA	13/13 (100.0%)	NA
	41-60	6/6 (100.0%)		9/9 (100.0%)	
		7/7 (100.0%)	NA	7/7 (100.0%)	NA
	Above 61	5/5 (100.0%)		1/1 (100.0%)	
		0/0 (00.0%)	NA	0/0 (00.0%)	NA
Nutritional Status (BMI)	Normal	9/9 (100.0%)		13/13 (100.0%)	
		7/7 (100.0%)	NA	7/7 (100.0%)	NA
	Overweight	2/2 (100.0%)		0/0 (00.0%)	
		2/2 (100.0%)	NA	2/2 (100.0%)	NA
	Underweight	10/10 (100.0%)		7/7 (100.0%	
		11/11 (100.0%)	NA	11/11 (100.0%)	NA
Socioeconomic Status	Low income	7/7 (100.0%)		8/8 (100.0%)	
		9/9 (100.0%)	NA	9/9 (100.0%)	NA
	Middleincom	6/6 (100.0%)		7/7 (100.0%)	
		5/5 (100.0%)	NA	5/5 (100.0%)	NA
	High income	8/8 (100.0%)		5/5 (100.0%)	
		6/6 (100.0%)	NA	6/6 (100.0%)	NA

Statistical test shows that less than 0.005 *P*-value is significant, more than 0.005 *P*-value is not significant and statistical test not applicable (NA).

In case of asthma, out of 20 patients, all the 20 (100%) have proved to be western blot positive and at the same time out

of 20 control subjects 20 (100%) were proved to be western blot positive. Statistically there is no significant correlation. Sex-wise, both male and female patients have shown 100% *H. pylori* infection, nevertheless statistics did not support any correlation between the two diseases. Age group-wise analysis also did not reveal any relation between the asthma and *H. pylori* infection. Results pertaining to nutritional status and socioeconomic status also did not reveal any significant correlation between the *H. pylori* incidence and asthma. Thus analysis of the results pertaining to demography (Table 2) revealed that absolutely there was no correlation between COPD, asthma and the seropositivity of *H. pylori*.

Testing of serum antibodies against the standard antigens showed that cagA (p120) antibodies were found in COPD, asthma as well as control subjects. Similarly, certain antibodies like p67, p57, p54, p50, p41 were found in detectable quantities in both types of patients and also healthy controls. Interestingly, antibodies against p33, p30, p29, p26, p19 and p17 which are considered to be positive for *H. pylori* were not observed in detectable quantities (Table 3). All in all, western blot investigations did not reveal any connectivity between COPD, asthma and *H. pylori* infection.

Table 3

IgG anti-*H. pylori* antigens find in obstractive airway diseases and control subjects by immunoblot test.

Western blot IgG antigen *H. pylori*	IgG-anti *H. pylori* COPD seropositivity (%)	IgG-anti *H. pylori* control seropositivity (%)	IgG-anti *H. pylori* asthma seropositivity (%)
P120, cagA	15/21 (71.4)	17/20 (85.0)	15/20 (75.0)
P95, vacA	1/21 (4.8)	1/20 (5.0)	1/20 (5.0)
p75	0/21 (0.0)	1/20 (5.0)	1/20 (5.0)
P67, Flag	12/21 (57.1)	12/20 (60.0)	10/20 (50.0)
P66, ureB	13/21 (61.9)	14/20 (70.0)	11/20 (55.0)
P57	18/21 (85.7)	15/20 (75.0)	14/20 (70.0)
P54	14/21 (66.7)	9/20 (45.0)	6/20 (30.0)
P50	10/21 (47.6)	13/20 (65.0)	14/20 (70.0)
P41	8/21 (38.1)	11/20 (55.0)	12/20 (60.0)
P33	0/21 (0.0)	4/20 (20.0)	2/20 (10.0)
P30	2/21 (9.5)	5/20 (25.0)	0/20 (0.0)
P29, ureA	9/21 (42.9)	9/20 (45.0)	3/20 (15.0)
P26	9/21 (42.9)	9/20 (45.0)	2/20 (10.0)
P19, omp	3/21 (14.3)	3/20 (15.0)	0/20 (0.0)
P17	0/21 (0.0)	1/20 (5.0)	0/20 (0.0)

Table 4 presents the results pertaining to *H. pylori* association with COPD and asthma as revealed by IIFA tests. A critical study of the table revealed that out of 27 subjects suffering with COPD, 19 (70.4%) have proved to be *H. pylori* positive, whereas out of 42 control subjects 4 (9.5%) have proved to be positive. Thus, there was a valid significance (P<0.000) between the association of *H. pylori* infection and COPD. In case of asthma, out of 20 patients, 7 (35.0%) have proved to be IIFA positive, whereas in control subjects out of 42, only 4 (9.5%) were proved to be positive. In this case also there was a significant correlation between the *H. pylori* infection and asthma. Demographic-wise analysis of IIFA results are presented in Table 4. Gender-wise statistically analysis revealed a highly significant correlation between *H. pylori* infection and COPD. In different age group people a significant correlation was also noticed, though there is some variation between two age groups. Relationship between nutritional status, COPD and *H. pylori* infection showed that in normal and underweight people there was a significant correlation between COPD and *H. pylori* infections. Among different socioeconomic status groups a high significance (P<0.000) was observed in low income and middle income groups. With regard to relationship between asthma and *H. pylori* infection a high significant correlation was observed for a nutritional status in case of normal and underweight people.

Thus, the IIFA investigations revealed a positive correlation between *H. pylori* infection and obstructive airway diseases (COPD and asthma).

Table 4

Demographic wise IgG anti-*H. pylori* association in obstractive airway diseases and control by IIFA test.

Demographic		COPD vs. control	Significance value	Asthma vs. control	Significance value
Gender	Male	14/17 (82.4%)		4/10 (40.0%)	
		2/21 (9.5%)	P<0.000	2/21 (9.5%)	P<0.045
	Female	5/10 (50.0%)		3/10 (30.0%)	
		2/21 (9.5%)	P<0.012	2/21 (9.5%)	P<0.147
	Total	19/27 (70.4%)		7/20 (35.0%)	
		4/42 (9.5%)	P<0.000	4/42 (9.5%)	P<0.014
Age group	20-40	6/11 (54.5%)		2/10 (20.0%)	
		3/27 (11.1%)	P<0.004	3/27 (11.1%)	P<0.482
	41-60	8/10 (80.0%)		5/09 (55.6%)	
		1/15 (6.7%)	P<0.000	1/15 (6.7%)	P<0.007
	Above 61	5/6 (83.3%)		0/0 (0.0%)	
		0/0 (0.0%)	NA	0/0 (0.0%)	NA
Nutritional Status (BMI)	Normal	7/10 (70.0%)		3/13 (23.1%)	
		0/4 (0.0%)	P<0.000	0/4 (0.0%)	P<0.000
	Overweight	1/1 (100.0%)		0/0 (0.0%)	
		0/3 (0.0%)	P<0.171	0/3 (0.0%	NA
	Underweight	11/15 (73.3%)		4/7 (57.1%)	
		4/25 (16.0%)	P<0.000	4/25 (16.0%)	P<0.000
Socioeconomic Status	Low income	5/9 (55.6%)		2/8 (25.0.%)	
		1/17 (5.9%)	P<0.004	1/17 (5.9%)	P<0.170
	Middle income	8/10 (80.0%)		3/7 (42.9%)	
		1/13 (7.7%)	P<0.000	1/13 (7.7%)	P<0.061
	High income	6/8 (75.0%)		2/5 (40.0%)	
		2/12 (16.7%)	P<0.009	2/12 (16.7%)	P<0.301

Statistical test shows that less than 0.005 P-value is significant, more than 0.005 P-value is not significant and statistical test not applicable (NA).

4. Discussion

In this study, we made an attempt to evaluate the possible relationship between *H. pylori* infection in COPD and asthma patients by ELISA, western blot and IIFA. The IIFA results suggested a significant association between *H. pylori* infection and COPD and asthma diseases. Previously, a number of investigators reported that *H. pylori* infection

might play a supporting role for COPD and asthma[11,14]. A small number of epidemiological and serologic case control studies suggest that patients with chronic obstructive pulmonary diseases have an increased seroprevalence of *H. pylori*[14]. On the other hand, bronchial asthma does not seem to be related to *H. pylori* infection[19]. Therefore, these investigations have carried out a prospective pilot study in a sample of 60 bronchitic patients and found an increased *H. pylori* seroprevalence (81.6% vs. 57.9% in controls). Moreover, for the first time they showed that *H. pylori* infection per se might be related to an increased risk of developing chronic bronchitis. A large epidemiological study in a Danish adult population showed that COPD might be much more prevalent in *H. pylori* immunoglobulin seropositive woman than in uninfected ones[20]. Our results are also similar to this observations. In order to further investigate the reported association, they performed two case control studies in the Greek population[21]. In the first they studied a cohort of 144 patients with chronic bronchitis and 120 control subjects and found that *H. pylori* seropositivity was significantly higher in patients than that in controls[12]. A more recent study by kanbay *et al*. concerning the *H. pylori* seroprevalence in a subgroup of COPD patients with chronic bronchitis confirmed above results[22]. They found that *H. pylori* seropositivity in bronchitic patients was significantly higher than that in controls. Moreover, Roussos *et al.* showed that *H. pylori* IgG levels might be correlated with the severity of COPD. The mechanisms underlying the suggested association between COPD and *H. pylori* infection are not clear[12]. Both *H. pylori* colonization of gastric mucosa and COPD development are related to old age, male sex and low socioeconomic status[23,24]. In all reviewed studies, COPD patients were well matched with control subjects for all these parameters. However, as *H. pylori* infection is usually acquired during childhood, matching for socioeconomic status should be performed for childhood and not for the time of study[25]. Therefore, inappropriate matching for socioeconomic status should be regarded as a limitation of all mentioned studies[12]. Cigarette smoking could be another confounding factor. It is well known that tobacco use represents the major cause of COPD[25]. On the other hand, data on the relation between *H. pylori* infection and smoking habits are controversial. A low, normal and high *H. pylori* prevalence in smokers has been reported[26-28]. Therefore, as the relation between tobacco use and *H. pylori* remains unclear, the possible impact of cigarette smoking on both COPD development and *H. pylori* infection should be regarded as a limitation of all reviewed studies. There are no studies in the literature focused on the potential etiopathogenetic role of *H. pylori* infection in COPD[14]. The primary evidence for an association between *H. pylori* infection and COPD rests on serologic case control studies[14]. In 1998, Tsang *et al.* found an increased *H. pylori* seroprevalence in patients with active bronchiectasis[29]. Hashemi *et al.* found a significant association between anti cagA IgG but not anti *H. pylori* IgG and COPD. In addition, they did not find any association between *H. pylori* infection and the severity of COPD[30]. A study conducted by Fullerton and his co-workers revealed no association between *H. pylori* serologic status and COPD, asthma allergic diseases and decline in lung function[31]. Tsang *et al.* while estimating the prevalence of *H. pylori* infection in a cohort of 90 patients with bronchial asthma concluded that bronchial asthma might not be associated with *H. pylori* infection[32]. A more recent study conducted by Jun *et al.* showed no significant association between mild asthma and *H. pylori* infection[33].

The present studies with ELISA revealed no association between COPD, asthma and *H. pylori* infection which are in agreement with the observations made by earlier investigators[13,34,35].

The data on western blot test revealed that diseased and healthy subjects have shown similar *H. pylori* status. Thus, this test revealed no relationship between *H. pylori* infection and COPD and asthma diseases. A survey of literature shows that no such investigations were made earlier.

In our IIFA study, anti-*H. pylori* against IgG antibodies in COPD versus control groups are 70.4% vs. 9.5% (P=0.000). In demographic study of gender group in male 82.4% vs. 9.5% (P=0.000), female 50.0% vs. 9.5% (P=0.012) and age group 20-40 54.5% vs. 11.1% (P=0.004), 41-60 80.0% vs. 6.7% (P=0.000) and BMI group wise in underweight 73.3% vs. 16.0% (P=0.000) and the IIFA tests revealed a strong relationship between *H. pylori* infection in COPD and a poor relationship between *H. pylori* infection and asthma. In this regard our studies are first of its kind and as such no reports are available for comparison purpose.

Out of the present investigations, it can be concluded that there is a correlation between *H. pylori* infection and obstructive airway diseases (COPD and asthma). However, this relationship can be confirmed only through IIFA investigations. Western blot and ELISA investigations are not helpful in this regard.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

H. pylori infection affects an approximately 50% of the world population and it has a well defined battery of virulence factors, which allow the organism to colonize the gastric mucosa, evade host defence and finally damage host tissue. Recent studies suggest an increased *H. pylori* prevalence in patients with various extra gastrointestinal disorders, including skin, cardiovascular, rheumatic and liver diseases. However, at present, there is no definite proof of a casual relationship between *H. pylori*, the two obstructive airway diseases, COPD and asthma diseases. In view of suspected and unconfirmed association of *H. pylori* in COPD and asthma, in the present investigations an attempt was made to establish the link, between these diseases through sero-techniques like ELISA, western blot and IIFA.

Research frontiers

Studies are being performed in order to establish the link between *H. pylori*, COPD, and asthma diseases through sero-techniques like ELISA, western blot and IIFA.

Related reports

The IIFA results suggest a significant association between *H. pylori* infection and COPD and asthma diseases. Previously, a number of investigators reported that *H. pylori* infection might play a supporting role for COPD and asthma. A small number of epidemiological and serologic case control studies suggest that patients with chronic obstructive pulmonary diseases have an increased sero prevalence of *H. pylori*. On the other hand, bronchial asthma does not seem to be related to *H. pylori* infection. Therefore, these investigations have carried out a prospective pilot study in a sample of 60 bronchitic patients and found an increased *H. pylori* seroprevalence (81.6% vs. 57.9% in controls). Moreover, for the first time they showed that *H. pylori* infection per se might be related to an increased risk of developing chronic bronchitis.

Innovations & breakthroughs

Among different socioeconomic status groups a high significance (P<0.000) was observed in low income and middle income groups. With regard to relationship between asthma and *H. pylori* infection a high significant correlation was observed for a nutritional status in case of normal and underweight people.

Applications

The present studies with ELISA revealed no association between COPD, asthma and *H. pylori* infection which are in agreement with the observations made by earlier investigators.

The data on western blot test revealed that diseased and healthy subjects have shown similar *H. pylori* status. Thus, this test revealed no relationship between *H. pylori* infection and COPD and asthma diseases. A survey of literature shows that no such investigations were made earlier.

Peer review

This is a good study in which authors evaluated the correlation between *H. pylori* infection and obstructive airway diseases (COPD and asthma). This relationship can be confirmed only through IIFA investigations. In this regard this study is first of its kind and no reports are available for comparison purpose.

References

[1] Porras C, Nodora J, Sexton R, Ferreccio C, Jimenez S, Dominguez RL, et al. Epidemiology of *Helicobacter pylori* infection in six Latin American countries. *Cancer Causes Control* 2013; **24**(2): 209-215.

[2] Liu YE, Gong YH, Sun LP, Xu Q, Yuan Y. The relationship between *H. pylori* virulence genotypes and gastric diseases. *Pol J Microbiol* 2012; **61**(2): 147-150.

[3] Blaser MJ. *Helicobacter pylori* and other gastric *Helicobacter* species. In: Mandell GL, Dolin JR, Bennett R, editors. Principles and practice of infectious diseases. Philadelphia: Elsevier; 2010, p. 2803-2813.

[4] Siva R, Birring SS, Berry M, Rowbottom A, Pavord ID. Peptic ulceration, *Helicobacter pylori* seropositivity and chronic obstructive pulmonary disease. *Respirology* 2013; **18**: 728-731.

[5] Doglioni C, Ponzoni M, Ferreri AJ, Savio A; Gruppo Italiano Patologi Apparato Digerente (GIPAD); Società Italiana di Anatomia Patologica e Citopatologia Diagnostica/International Academy of Pathology, Italian division (SIAPEC/IAP). Gastric lymphoma: the histology report. *Dig Liver Dis* 2011; **43**: S310-S318.

[6] Chiba T, Marusawa H, Matsumoto Y, Takai A. [Chronic inflammation and gastric cancer development]. *Nihon Rinsho*. 2012; **70**(10): 1694-1698. Japanese.

[7] Salem EM, Yar T, Bamosa AO, Al-Quorain A, Yasawy MI, Alsulaiman RM, et al. Comparative study of *Nigella sativa* and triple therapy in eradication of *Helicobacter pylori* in patients with non-ulcer dyspepsia. *Saudi J Gastroenterol* 2010; **16**(3): 207-214.

[8] Banić M, Franceschi F, Babić Z, Gasbarrini A. Extragastric manifestations of *Helicobacter pylori* infection. *Helicobacter* 2012; **17**: 49-55.

[9] Talebi-Taher M, Mashayekhi M, Hashemi MH, Bahrani V.

Helicobacter pylori in diabetic and non-diabetic patients with dyspepsia. *Acta Med Iran* 2012; **50**(5): 315-318.

[10] Hosseini E, Poursina F, de Wiele TV, Safaei HG, Adibi P. *Helicobacter pylori* in Iran: A systematic review on the association of genotypes and gastroduodenal diseases. *J Res Med Sci* 2012; **17**(3): 280-292.

[11] Malfertheiner MV, Kandulski A, Schreiber J, Malfertheiner P. *Helicobacter pylori* infection and the respiratory system: a systematic review of the literature. *Digestion* 2011; **84**(3): 212-220.

[12] Gencer M, Ceylan E, Yildiz Zeyrek F, Aksoy N. *Helicobacter pylori* seroprevalence in patients with chronic obstructive pulmonary disease and its relation to pulmonary function tests. *Respiration* 2007; **74**(2): 170-175.

[13] Manolakis A, Kapsoritakis AN, Potamianos SP. A review of the postulated mechanisms concerning the association of *Helicobacter pylori* with ischemic heart disease. *Helicobacter* 2007; **12**(4): 287-297.

[14] Roussos A, Philippou N, Mantzaris GJ, Gourgoulianis KI. Respiratory diseases and *Helicobacter pylori* infection: is there a link? *Respiration* 2006; **73**: 708-714.

[15] Blecker U, Lanciers S, Hauser B, Vandenplas Y. Diagnosis of *Helicobacter pylori* infection in adults and children by using the Malakit *Helicobacter pylori*, a commercially available enzyme-linked immunosorbent assay. *J Clin Microbiol* 1993; **31**: 1770-1773.

[16] Bolin I, Lonroth H, Svennerhole AM. Identification of *Helicobacter pylori* by immunological dot blot method based on reaction of a species-specific monoclonal antibody with a surface-exposed protein. *J Clin Microbiol* 1995; **33**: 381-384.

[17] Krausse R, Garten L, Harder T, Ullmann U, Birkner T, Doniec M, et al. Clinical relevance of CagA-specific antibodies related to CagA status of *Helicobacter pylori* isolates using immunofluorescence test and PCR. *Infection* 2001; **29**: 154-158.

[18] Allerberger F, Oberhuber G, Wrba F, Puspok A, Dejaco C, Dierich MP. Detection of *Helicobacter pylori* infection using single serumspecimens: comparison of five commercial serological tests. *Hepatogastroenterology* 1996; **43**: 1656-1659.

[19] Kurtaran H, Uyar ME, Kasapoglu B, Turkay C, Yilmaz T, Akcay A, et al. Role of *Helicobacter pylori* in pathogenesis of upper respiratory system diseases. *J Natl Med Assoc* 2008; **100**: 1224-1230.

[20] Rosenstock SJ, Jorgensen T, Andersen LP, Bonnevie O. Association of *Helicobacter pylori* infection with lifestyle, chronic disease, body-indices, and age at menarche in Danish adults. *Scand J Public Health* 2000; **28**: 32-40.

[21] Yula E, Nagiyev T, Kaya OA, Inci M, Celik MM, Köksal F. Detection of primary clarithromycin resistance of *Helicobacter pylori* and association between cagA (+) status and clinical outcome. *Folia Microbiol (Praha)* 2013; **58**(2): 141-146.

[22] Kanbay M, Gur G, Akcay S, Yilmaz U. *Helicobacter pylori* seroprevalence in patients with chronic bronchitis. *Respir Med* 2005; **99**: 1213-1216.

[23] Desai CS, Colangelo LA, Liu K, Jacobs DR Jr, Cook NL, Lloyd-Jones DM, et al. Prevalence, prospective risk markers, and prognosis associated with the presence of left ventricular diastolic dysfunction in young adults: the coronary artery risk development in young adults study. *Am J Epidemiol* 2013; **177**(1): 20-32.

[24] Wang HM, Lo GH, Chen WC, Hsu PI, Yu HC, Lin CK, et al. Efficacy of transient elastography in screening for large esophageal varices in patients with suspicious or proven liver cirrhosis. *J Dig Dis* 2012; **13**(8): 430-438.

[25] Toelle BG, Xuan W, Bird TE, Abramson MJ, Atkinson DN, Burton DL, et al. Respiratory symptoms and illness in older Australians: the burden of obstructive lung disease (BOLD) study. *Med J Aust* 2013; **198**(3): 144-148.

[26] Shi Y, Gong H, Zhou L, Tao L, Shi Y, Cao W, et al. Association between *Helicobacter pylori* infection and laryngeal squamous cell carcinoma in a Chinese male population. *ORL J Otorhinolaryngol Relat Spec* 2011; **73**(6): 295-300.

[27] Zhang Y, Sun LP, Xing CZ, Xu Q, He CY, Li P, et al. Interaction between GSTP1 Val allele and *H. pylori* infection, smoking and alcohol consumption and risk of gastric cancer among the Chinese population. *PLoS One* 2012; doi: 10.1371/journal.pone.0047178.

[28] Goenka MK, Majumder S, Sethy PK, Chakraborty M. *Helicobacter pylori* negative, non-steroidal anti-inflammatory drug-negative peptic ulcers in India. *Indian J Gastroenterol* 2011; **30**(1): 33-37.

[29] Tsang KW, Lam SK, Lam WK, Karlberg J, Wong BC, Hu WH, et al. High seroprevalence of *Helicobacter pylori* in active bronchiectasis. *Am J Respir Crit Care Med* 1998; **158**: 1047-1051.

[30] Hashemi SH, Nadi E, Hajilooi M, Seif-Rabiei MA, Roustaei U. Relationship between *Helicobacter pylori* Infection and chronic obstructive pulmonary disease. *Acta Med Iran* 2011; **49**(11): 721-724.

[31] Fulletron D, Britton JR, Lewis SA, Pavord ID, McKeever TM, Fogarty AW. *Helicobacter pylori* and lung function, asthma, atopy and allergic disease: a population-based cross-sectional study in adults. *Int J Epidemiol* 2009; **38**(2): 419-426.

[32] Tsang KW, Lam WK, Chan KN, Hu W, Wu A, Kwok E, et al. *Helicobacter pylori* sero-prevalence in asthma. *Respir Med* 2000; **94**: 756-759.

[33] Jun ZJ, Lei Y, Dobashi K, Mori M. *Helicobacter pylori* seroprevalence in patients with mild asthma. *Tohoku J Exp Med* 2005; **207**(4): 287-291.

[34] Reibman J, Marmor M, Filner J, Fernandez-Beros ME, Rogers L, Perez-Perez GI, et al.Asthma is inversely associated with *Helicobacter pylori* status in an urban population. *PloS One* 2008; doi: 10.1371/journal.pone.0004060.

[35] Minov JB, Karadzinska-Bislimovska J, Vasilevska K, Risteska-Kuc S, Stoleski S, Mijakoski D. The impact of *Helicobacter pylori* infection on lung function and severity of bronchial hyperresponsiveness in subjects with allergic asthma. *Am J Immunol* 2011; **7**: 62-67.