Research Article

Detection of *Helicobacter Pylori* in Bronchoalveolar Lavage of Patients With Chronic Obstructive Pulmonary Disease by Real Time Polymerase Chain Reaction

Mitra Samareh Fekri [1]; Seyed Mehdi Hashemi Bajgani [2]; Atefe Rasti [1,*]; Rostam Yazdani [2]; Hamid Reza Mollaie [2]

[1]Physiology Research Center (PRC), Kerman University of Medical Sciences, Kerman, IR Iran
[2]Afzalipour Hospital, Kerman University of Medical Sciences, Kerman, IR Iran

Corresponding author: Atefe Rasti, Physiology Research Center (PRC), Kerman University of Medical Sciences, Kerman, IR Iran. Tel: +98-9177108194, Fax: +98-3432264097, E-mail: atefe.rasti89@gmail.com-

Background: Chronic obstructive pulmonary disease (COPD) is one of the most important causes of disability and mortality in the world. Although cigarette smoking and environmental pollutants have been recognized as the major causes of COPD, the role of infection in the pathogenesis and progression of COPD has also been reported.

Objectives: The aim of the present study was to find the relationship between *Helicobacter Pylori* infection and COPD through anti *H. pylori* IgG serology, real time PCR of bronchoalveolar lavage and trans bronchial biopsy urease tests.

Patients and Methods: This descriptive cross-sectional study was carried out on 60 adults with COPD. After obtaining the patient's history, physical examination, spirometry and confirmation of COPD diagnosis by pulmonologist, subjects were selected through convenience sampling. In order to determine the severity and prognosis of disease, the global initiative for chronic obstructive lung disease (GOLD) criteria and BODE index were used. Subjects underwent bronchoscopy for obtaining bronchoalveolar lavage (BAL) samples and biopsy was performed. Biopsy and BAL samples were investigated respectively by urease test and real time PCR. Moreover, patients' serum samples were serologically studied for detection of anti *H. pylori* IgG.

Results: Mean age of the participants was 60.65 ± 9.15 years, and 25% were female and 75% were male. The prevalence rate of *H. pylori* in COPD patients was 10% according to real time PCR, 88.3% according to the serology test and 0% based on the urease test. According to the results of PCR and considering the severity of disease based on the GOLD criteria, from those with a positive PCR, one patient (16.6%) had very severe obstruction, three (50%) had severe obstruction and two patients (33.3%) had moderate obstruction. The relationship between *H. pylori* presence (based on PCR) and disease severity and prognosis was not statistically significant.

Conclusions: These findings can justify the hypothesis of direct injury and chronic inflammation via inhalation and aspiration resulting in *H. pylori* colonization. In fact, it is thought that *H. Pylori* infection, beside the host genetic vulnerability and other environmental risk factors might make the patient susceptible to COPD or lead to COPD worsening. Although we found *H. pylori* infection in some patients with COPD, the results of this study, could not explain the pathogenic mechanisms of COPD.

Keywords: Chronic Obstructive Pulmonary Disease; *Helicobacter pylori*; Real Time PCR; Serology; Urease

1. Background

Chronic obstructive pulmonary disease (COPD) is one of the most important causes of disability and mortality in the world. It causes physical disability, affects quality of life and causes death. Chronic obstructive pulmonary disease is the fourth cause of death in the United States of America (1). According to the global initiative for chronic obstructive lung disease (GOLD), COPD is defined as a preventable and treatable disease that leads to the narrowing of the airways and is not fully reversible (2). In industrial countries, cigarette smoking and in developing countries, cigarette smoking and environmental pollutants such as floating particles due to cooking in nonstandard environments are the most common causes of COPD (3). In addition to the role of cigarette, environmental pollutants and genetic factors, the role of infections have also been mentioned in the pathogenesis and progression of this disease (4).

Helicobacter pylori is a Gram-negative, microaerophilic bacterium with a slow growing nature. The most important biochemical index of *H. pylori* is the production of large quantities of urease. This bacterium colonizes the gastric mucosa and causes a long-term immune response and local and systemic inflammation in the host. They affect the gastric mucosa of almost 50% of the world's population (5-7). Previous studies have provided extensive evidence on the role of *H. pylori* in chronic gastritis, peptic ulcer, B-cell lymphoma of gastric mucosa associated-lymphoid tissue (MALT) lymphoma and gastric cancer (diffuse, intestinal) (8-10).

Recent studies have shown that there is a relationship between *H. pylori* infection and extra gastric diseases such as cardiovascular, skin, liver and rheumatic diseases (11, 12). This bacterium is associated with several respiratory diseases such as COPD, bronchiectasis, asthma, lung cancer and lung tuberculosis (13-15). Indeed, before identifying *H. pylori*, higher incidence of COPD and tuberculosis had been observed in patients with peptic ulcers compared to the normal population (16-20). Release of similar inflammatory cytokines in *H. pylori* infection and acute COPD attacks has been proved by some studies (16, 17, 21). The probable mechanism for the relationship between *H. pylori* and lung diseases might be the systemic effect of gastrointestinal peptides (gastrin, somatostatin) and cytokine release or direct injury and chronic inflammation of airways due to aspiration and inhalation. Since the release of inflammatory cytokines including IL1β, IL8 and TNFα is observed in chronic lung diseases and *H. pylori* eradication causes returning of these cytokines to the normal levels (22), it seems that activation of inflammatory mediators by *H. pylori* is the pathogenic mechanism of extragastric manifestations of *H. pylori* infection (22-25).

2. Objectives

The present study was designed to investigate the relationship between *H. pylori* and COPD via anti *H. pylori* IgG serology, real-time PCR of bronchoalveolar lavage and transbronchial biopsy urease tests.

3. Patients and Methods

This descriptive cross-sectional study was performed on 60 adults with COPD, who had been referred to our lung diseases diagnosis department in 2012. After obtaining history, physical examination, spirometry and confirmation of COPD diagnosis by the pulmonologist, subjects were selected through convenience sampling. The rate of airway obstruction in COPD patients was determined based on the GOLD criteria (Global Initiative for Obstructive Lung Diseases):

Grade 0: Patients at risk with normal spirometry

Grade 1: Mild obstruction, FEV1/FVC < 70%, FEV1 ≥ 80%

Grade 2: Moderate obstruction, FEV1/FVC < 70, 50% ≥ FEV1<80%

Grade 3: Severe obstruction, FEV1/FVC < 70%, 30% ≥ FEV1 < 50%

Grade 4: Very severe obstruction, FEV1/FVC < 70%, FEV1 < 30% or FEV1 < 50%, associated with respiratory failure and symptoms of right-sided heart failure (2).

After explaining the research goals and details to the subjects and obtaining their informed written consent, they were enrolled in the study. The study was approved by Kerman University of Medical Sciences (code: 91/218). All patients underwent spirometry. In order to determine the severity of lung chronic obstruction, the GOLD criteria, and for determining the severity and prognosis of disease, the BODE-index, were used. Four factors were used for the evaluation of the severity of obstruction based on the BODE-Index including, weight (BMI), airflow obstruction (FEV1), dyspnea and exercise capacity (six-minute walking distance), that in comparison to FEV1 provide more accurate information about the rate of obstruction and the disease prognosis (26, 27).

Demographic data including age, sex, cigarette smoking, opium consumption and educational level were recorded and symptoms of reflux including regurgitation, heartburn, epigastric pain, dysphonia and dysphagia were recorded based on the gastroesophageal reflux disease (GERD) questionnaire (28). Those who were in an exacerbation condition of COPD and had a history of *H. pylori* eradication and also had severe hypoxia and cardiac arrhythmia were excluded from the study. All subjects underwent bronchoscopy and bronchoalveolar lavage (BAL) and biopsy sampling of the airways. Biopsy samples were studied using the urease test, the BAL sample was studied by Real time PCR and serum was serologically examined for detection of anti *H. pylori* IgG.

3.1. Serological Test

The serum samples were kept at -20°C and used for measuring anti *H. pylori* IgG by the enzyme linked immunosorbent assay (ELISA) method (Trinity kit, Germany). Samples with IgG ≥ 1.2 immune status ratio (ISR) were considered positive according to the manufacturer's instructions.

3.2. DNA Extraction

After collecting BAL samples, they were kept at -80°C until use in the experiments. Then, *H. pylori* DNA was extracted using the QIAamp DNA Mini kit (Qiagen, Germany), according to the manufacturer's instructions. Extracted DNA pellets were re-suspended in 100 μL of pre-warmed elution buffer and stored at -20°C until use.

3.3. Molecular Test

A SYBR Green Real Time PCR assay was developed to screen DNA for *H. pylori*. The primers amplify a 102 bp region for detection of UreA (Urease Gene) in *H. pylori* and a 145 bp region for detection of pathogenic *H. pylori*. The urease gene of *H. Pylori* was used in order to detect the presence of this bacterium and CagA was used for identification of the pathogenic strains of the bacterium. We used the β-Actin housekeeping gene for the internal control gene and for normalizing the test. PCR reactions contained 5 μL of DNA, 100 ng of each primer, and 14 μL of Maxima SYBR Green PCR Master Mix (Thermo Scientific) in a 20 μL total volume. Thermo cycling conditions were as follows: 95°C for 10 minutes, 40 cycles at 95°C for 15 seconds, and 60°C for 40 seconds; quantitative determination of the amplified products was done with the Rotor Gene 6000 (Corbett Research, Australia). The PCR results were considered positive when copy/mL ≥ 10 (29-31). Sequence of primers has been shown in Table 1.

Table 1. Sequence of Primers Used in This Study

Name of Gene	Forward Primer	Reverse Primer
UreA	GAGAATGAGATGAAACTCACCC	TTGTCTGCTTGTCTATCAACC3
CagA	CTCATTGCGAAGGCGACCT	TCTAATCCTGTTTGCTCCCCA
β-actin	TGCCTATCAGAAAGTGGTGGCT	GCTCAAGGCCCTTCATAATATCC

3.4. Urease Test

Rapid Urease Test was performed on bronchial samples by the Man Company Kit (Iran) and based on the producer manual; accordingly, 1 mL of the prepared solution was poured into a cup and the biopsy sample was placed in this solution for 60 minutes. Color change from yellow to pink-purple was considered as *H. pylori* positive; this occurs due to ammonium production by the bacterium.

4. Results

The mean age of participants was 60.65 ± 9.15 years (age range: 40-86). In total, 15 individuals (25%) were female and 45 (75%) were male. From all participants, 36 (60%) were cigarette smokers and 41 (68.3%) were opium addicts. In regards to the severity of disease based on FEV1, seven patients (11.66%) had mild obstruction, 29 (48.34%) had moderate obstruction, 15 (25%) had severe obstruction and 9 (15%) had very severe obstruction. The prevalence of *H. pylori* in COPD patients was 10% (6 of 60 patients) based on real-time PCR, 88.3% (53 of 60 patients) based on serology and 0% based on the urease test (Figure 2).

For determining the relationship between *H. Pylori* (based on urease test) and disease severity, urease tests were obtained from all patients, and the results were negative for all subjects. The relationship between *H. pylori* (based on serology) and disease severity was also determined based on the GOLD criteria; in the group that were seropositive based on the enzyme linked immunosorbent (ELISA) method, five (9.4%) had mild obstruction, 27 (50.9%) had moderate obstruction, 13 (24.5%) had severe obstruction and eight (15.1%) suffered from very severe obstruction. No significant relationship was found between the presence of *H. pylori* (based on serology) and disease severity (P = 0.907). The results are shown in Table 2 and Figure 1.

According to the results of real-time PCR and considering the severity of disease based on the GOLD criteria, amongst those with positive PCR results, one patient (16.6%) had very severe obstruction, three (50%) had severe obstruction and two (33.3%) had moderate obstruction. In other patients real-time PCR results were negative. The relationship between presence of *H. pylori* (based on real-time PCR) and disease severity was not statistically significant (P = 0.808). The results are demonstrated in Table 3 and Figure 1.

In order to increase the accuracy of determining prognosis of the disease, the BODE index was also used. Accordingly, based on PCR, BODE Index of four patients (66.7%) was between three and four, one patient (14.3%) had a score of six and one patient (16.7%) scored eight. Based on seropositivity, BODE score of 38 patients (71.7%) was between three and four, 10 (18.9%) scored from five to six and five (9.4%) scored from seven to nine; a higher BODE Index correlates with an increasing risk of death. No significant relationship was found between *H. pylori* infection (based on real-time PCR and serology) and BODE index (P = 0.475 and P = 0.695, respectively). The results are shown in Tables 2 and 3.

Based on the urease, serology and real-time PCR tests and logistic regression, no significant relationship was found between *H. pylori* in COPD patients and variables of age, educational level, cigarette smoking and reflux symptoms. Based on the serology test, logistic regression showed a significant relationship only with dysphagia (P = 0. 035) (Table 1). Moreover, based on real-time PCR of opium addicts, both raw test and logistic regression showed a significant relationship (P = 0.041) (Table 3) (Figure 2).

Table 2. The Relationship Between Presence of *H. pylori* in COPD Patients and BODE Index, FEV1 and Other Parameters (Based on Serology)

| Serology	Crude				Adjusted			
	B	S.E	P Value [a]	OR	B	S.E	P Value	OR [b]
BODE index	-0.129	0.584	0.826	0.879	0.412	1.051	0.695	1.511
FEV$_1$	0.495	0.359	0.167	1.64	-0.07	0.609	0.907	0.931
Age	-0.020	0.044	0.644	0.980	-0.04	0.062	0.497	0.957
Cigarette smoking	0.001	0.003	0.820	1.001	0.00	0.003	0.915	1.00
Opium consumption	0.127	0.894	0.887	1.13	0.139	0.886	0.876	1.149
Hoarseness	0.327	0.961	0.733	1.38	1.06	0.818	1.93	2.90
Heartburn	1.36	1.11	0.232	3.90	1.69	0.886	0.056	5.44
Regurgitation	-0.657	1.08	0.545	0.518	0.087	0.812	0.915	1.09
Dysphasia	0.943	0.985	0.338	2.56	1.76	0.840	0.035	5.86
Epigastria pain	0.950	0.983	0.333	2.58	1.43	0.827	0.083	4.20

[a] Based on logistic regression.
[b] B(Estimated parameter coefficient), SE (Standard Error), OR(Odds Ratio), FEV1(Forced Expiratory Volume in one second)

Table 3. The Relationship Between Presence of *H. pylori* in COPD Patients and BODE Index, FEV1 and Other Parameters (Based on Real Time PCR)

PCR	Crude				Adjusted			
	B	**S.E**	**P Value** [a]	**OR**	**B**	**S.E**	**P Value**	**OR**
BODE index	-0.129	0.584	0.082	0.879	-0.064	0.907	0.475	0.052
FEV$_1$	-0.208	0.418	0.619	0.812	-0.173	0.714	0.808	0.084
Age	-0.009	-0.044	0.844	0.991	-0.015	0.068	0.820	0.985
Cigarette smoking	0.000	0.003	0.900	1.00	-0.001	0.003	0.788	0.999
Opium consumption	-2.420	1.170	0.038	0.088	-2.36	1.15	0.041	0.094
Hoarseness	-0.351	1.030	0.733	1.42	0.315	0.957	0.742	1.37
Heartburn	0.402	1.166	0.730	0.669	0.154	0.955	0.872	1.16
Regurgitation	-0.859	1.070	0.425	2.36	-0.811	0.953	0.395	2.25
Dysphasia	0.389	1.260	0.758	0.67	0.214	1.162	0.854	1.77
Epigastria pain	0.542	1.110	0.628	1.71	0.575	0.962	0.550	1.37

[a] Based on logistic regression.
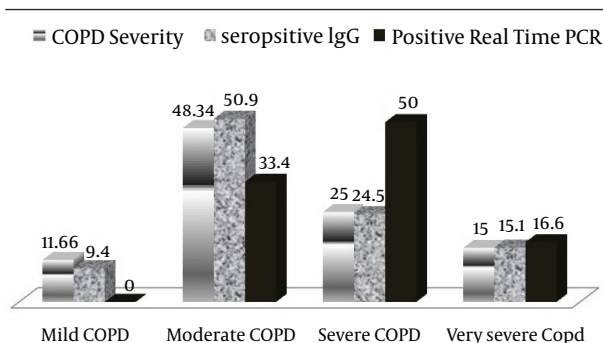
Figure 1. Chronic Obstructive Pulmonary Disease Patient Characteristics
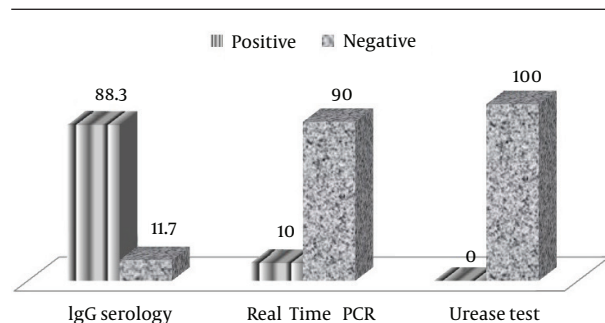
Figure 2. Prevalence of *H. pylori* in COPD Based on Urease Real Time PCR and Serological Tests

5. Discussion

In the present study, bronchoalveolar fluid of 10% of COPD patients was positive for *H. pylori* according to real time PCR and 88.3% of patients had positive results according to their serology test. Urease test results were negative for all patients. However, there was no signifi-

cant relationship between the obtained results and the severity and prognosis of COPD based on the GOLD and BODE index criteria. The relationship between chronic bronchitis and peptic ulcer was determined several years prior to the identification of *H. pylori*, and the prevalence of chronic bronchitis in patients with peptic ulcer was shown to be two to three times more than the control group (18, 32). In a study performed during 1998, the prevalence of *H. pylori* seropositivity in patients with chronic bronchitis was more than that of the control group (81.6% vs. 57.9%) (33). A study in Denmark demonstrated an association between *H. pylori* IgG seropositivity and chronic bronchitis (34). According to this study, the prevalence of *H. pylori* IgG and CagA protein seropositivity in COPD patients was significantly higher than the control group and there was no significant correlation between seropositivity and pulmonary function test (35). It has also been reported that cases with positive IgG *H. pylori* antibody are more likely to be among chronic bronchitis patients (36). The findings of these studies are similar to our results.

In another previous study, Anti-Cag A level was significantly higher in the COPD group compared to the control group and, similar to our study, no significant relationship was found between *H. pylori* infection and COPD severity (37). In a study on the relationship between *H. pylori* infection and pulmonary function based on FEV1 and FVC, lower pulmonary function was reported in seropositive patients, yet after statistical adjustment for socioeconomic status, this relationship disappeared (38). In our study, also, no significant relationship was found between *H. pylori* seropositivity and pulmonary function tests. Furthermore, in one study that compared non-smoking male patients with non-smoking males without chronic respiratory disease, a strong relationship was found between COPD and *H. pylori* serology, yet, like our

study, no significant relationship was found between *H. pylori* seropositivity and pulmonary function tests (39). According to a study on the serum of 49 patients with chronic obstructive pulmonary disease, *H. pylori* IgG level in COPD patients was significantly higher than the control group and there was a significant correlation between the severity of the disease and *H. pylori* seropositivity (13). The rate of *H. pylori* seropositivity in our patients was 88.3% and there was no significant correlation between the severity of the disease and seropositivity; the difference between our and the previous study might be due to different sample sizes. A recent study showed that both peptic ulcer and positive *H. pylori* serology in COPD patients are more than the control group (40).

There are very limited studies that have performed a direct isolation of this bacterium from lung tissue. In one of the previous studies, 34 patients with various types of lung diseases underwent bronchoscopy and biopsy of lung tissue. Histopathology and urease test showed no positive evidence of *H. pylori* presence in biopsy samples (41). Similarly, in the present study, none of the urease tests were found positive in regards to *H. pylori*. In some previous studies on patients with bronchiectasis, BAL and lung tissue were investigated by PCR for the presence of *H. pylori* and, DNA of this bacterium was not found in any of the samples (42, 43). However, in the present study, the real-time PCR detected DNA of *H. pylori* bacterium in BAL of 10% of COPD patients.

All previous studies have been based on serological tests, however there are several intervening factors that affect the performance of these tests. Moreover, Iran is among regions with high prevalence of *H. pylori* and approximately 90% of adults in Iran are infected with this bacterium (44). In addition, factors such as age, sex and socioeconomic conditions are involved in COPD and *H. pylori* infection. Therefore, socioeconomic status should surely be considered in this regard. Indeed, cigarette smoking is also an intervening factor and is one of the major causes of COPD. In regards to the relationship between *H. pylori* infection and cigarette smoking, there are controversies amongst previous findings (15, 37). In the present study, there was no significant relationship between cigarette smoking and *H. pylori* in COPD patients.

5.1. Study Limitations

Chronic obstructive pulmonary disease patients, due to sputum production and many gastrointestinal problems frequently use antibiotics and anti-acids and since consumption of these medicines can affect the study results, they should be considered as part of patient's history. Further studies with a greater sample size and more severe cases of COPD are recommended, because in studies with more severe COPD patients, the number of positive cases in PCR might be higher, as the present study, revealed that positive cases in real time PCR were those with more severe COPD. Another limitation of the present study was the lack of a control group, yet since bronchoscopy is an invasive method, including a control group was ethically impossible.

In the present study, DNA of *H. pylori* was detected in about 10% of COPD patients by BAL real time PCR and 88.3% of patients were IgG seropositive. As previously mentioned, most previous studies had been based on serology. However, in the present study, in addition to serological tests, real time PCR was applied. The findings can explain the hypothesis of direct injury and chronic inflammation through inhalation and aspiration in addition to systemic immune response resulted from *H. pylori* colonization.

In fact, it is thought that *H. pylori* along with the host genetic susceptibility and other environmental risk factors can make the person susceptible to COPD occurrence or lead to COPD worsening. Although we found *H. pylori* infection in some patients with chronic obstructive pulmonary disease, the results of this study, could not explain the pathogenic mechanisms in chronic obstructive pulmonary disease. It is recommended for other researches to measure inflammatory factors such as IL-1, INFγ and IL8 in BAL of COPD patients who are *H. pylori* positive based on real time PCR of BAL, in order to identify pathogenic mechanisms of the relationship of this bacterium with COPD. Indeed, studies on genetic loci, through identifying human leukocyte antigens (HLAs), which can make the individual vulnerable to both *H. pylori* infection and COPD, are recommended.

Authors' Contributions

Mitra Samareh Fekri: designed and coordinated the study, participated in most of the experiments and prepared the manuscript. Mitra Samareh Fekri, Seyed Mehdi Hashemi Bajgani, Atefe Rasti and Rostam Yazdani: provided assistance for the design of the study, coordinated and carried out all the experiments and participated in the manuscript preparation. All authors have read and approved the content of the manuscript.

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References

1. Buist AS, McBurnie MA, Vollmer WM, Gillespie S, Burney P, Mannino DM, et al. International variation in the prevalence of COPD (the BOLD Study): a population-based prevalence study. *Lancet.* 2007;**370**(9589):741–50.
2. Committee GE. *Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease.*; 2009.
3. Dennis RJ, Maldonado D, Norman S, Baena E, Martinez G. Woodsmoke exposure and risk for obstructive airways disease among women. *Chest.* 1996;**109**(1):115–9.
4. Pauwels RA, Buist AS, Ma P, Jenkins CR, Hurd SS, Gold Scientific Committee . Global strategy for the diagnosis, management, and

prevention of chronic obstructive pulmonary disease: National Heart, Lung, and Blood Institute and World Health Organization Global Initiative for Chronic Obstructive Lung Disease (GOLD): executive summary. *Respir Care.* 2001;**46**(8):798–825.

5. Dixon MF, Genta RM, Yardley JH, Correa P. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol.* 1996;**20**(10):1161–81.

6. Mitchell H, Megraud F. Epidemiology and diagnosis of Helicobacter pylori infection. *Helicobacter.* 2002;**7 Suppl 1**:8–16.

7. Blaser MJ. Helicobacter pylori and other gastric Helicobacter species. In: Mandell GL, Dolin JR, Bennett R editors. *Principles and Practice of Infectious Diseases..* Philadelphia: Churchill Livingstone; 2010. pp. 2803–13.

8. Cohen H. Peptic Ulcer and Helicobacter Pylori. *GastroenterolClin North Am.* 2000;**29**(4):775–84.

9. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, et al. Helicobacter pylori infection and gastric lymphoma. *N Engl J Med.* 1994;**330**(18):1267–71.

10. Xue FB, Xu YY, Wan Y, Pan BR, Ren J, Fan DM. Association of H. pylori infection with gastric carcinoma: a Meta analysis. *World J Gastroenterol.* 2001;7(6):801–4.

11. Sherman PM, Lin FY. Extradigestive manifestation of Helicobacter pylori infection in children and adolescents. *Can J Gastroenterol.* 2005;**19**(7):421–4.

12. De Koster E, De Bruyne I, Langlet P, Deltenre M. Evidence based medicine and extradigestive manifestations of Helicobacter pylori. *Acta Gastroenterol Belg.* 2000;**63**(4):388–92.

13. 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):170–5.

14. Roussos A, Philippou N, Mantzaris GJ, Gourgoulianis KI. Respiratory diseases and Helicobacter pylori infection: is there a link? *Respiration.* 2006;**73**(5):708–14.

15. 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):212–20.

16. Nelson S, Summer WR, Mason CM. The role of the inflammatory response in chronic bronchitis: therapeutic implications. *Semin Respir Infect.* 2000;**15**(1):24–31.

17. Silva JR, Jones JA, Cole PJ, Poulter LW. The immunological component of the cellular inflammatory infiltrate in bronchiectasis. *Thorax.* 1989;**44**(8):668–73.

18. Kellow JE, Tao Z, Piper DW. Ventilatory function in chronic peptic ulcer. A controlled study of ventilatory function in patients with gastric and duodenal ulcer. *Gastroenterology.* 1986;**91**(3):590–5.

19. Lundegardh G, Helmick C, Zack M, Adami HO. Mortality among patients with partial gastrectomy for benign ulcer disease. *Dig Dis Sci.* 1994;**39**(2):340–6.

20. Huang SL, Su CH, Chang SC. Tumor necrosis factor-alpha gene polymorphism in chronic bronchitis. *Am J Respir Crit Care Med.* 1997;**156**(5):1436–9.

21. Keatings VM, Collins PD, Scott DM, Barnes PJ. Differences in interleukin-8 and tumor necrosis factor-alpha in induced sputum from patients with chronic obstructive pulmonary disease or asthma. *Am J Respir Crit Care Med.* 1996;**153**(2):530–4.

22. Kountouras J, Boura P, Lygidakis NJ. Omeprazole and regulation of cytokine profile in Helicobacter pylori-infected patients with duodenal ulcer disease. *Hepatogastroenterology.* 2000;**47**(35):1301–4.

23. Mitz HS, Farber SS. Demonstration of Helicobacter pylori in tracheal secretions. *J Am Osteopath Assoc.* 1993;**93**(1):87–91.

24. Mehmet N, Refik M, Harputluoglu M, Ersoy Y, Aydin NE, Yildirim B. Serum and gastric fluid levels of cytokines and nitrates in gastric diseases infected with Helicobacter pylori. *New Microbiol.* 2004;**27**(2):139–48.

25. Romero Adriàn T, Leal Montiel J. *Helicobacter pylori* Infection: Regulatory T Cells and Their Participation in the Immune Response. *Jundishapur J Microbiol.* 2013;**6**(4):5183.

26. Cote CG, Pinto-Plata VM, Marin JM, Nekach H, Dordelly LJ, Celli BR. The modified BODE index: validation with mortality in COPD. *Eur Respir J.* 2008;**32**(5):1269–74.

27. Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, et al. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. *N Engl J Med.* 2004;**350**(10):1005–12.

28. Jones R, Junghard O, Dent J, Vakil N, Halling K, Wernersson B, et al. Development of the GerdQ, a tool for the diagnosis and management of gastro-oesophageal reflux disease in primary care. *Aliment Pharmacol Ther.* 2009;**30**(10):1030–8.

29. He Q, Wang JP, Osato M, Lachman LB. Real-time quantitative PCR for detection of Helicobacter pylori. *J Clin Microbiol.* 2002;**40**(10):3720–8.

30. Kobayashi D, Eishi Y, Ohkusa T, Suzuki T, Minami J, Ishige., et al. Gastric mucosal density of Helicobacter pylori estimated by real-time PCR compared with results of urea breath test and histological grading. *J Med Microbiol.* 2002;**51**(4):305–11.

31. Rinttila T, Kassinen A, Malinen E, Krogius L, Palva A. Development of an extensive set of 16S rDNA-targeted primers for quantification of pathogenic and indigenous bacteria in faecal samples by real-time PCR. *J Appl Microbiol.* 2004;**97**(6):1166–77.

32. Arora OP, Kapoor CP, Sobti P. Study of gastroduodenal abnormalities in chronic bronchitis and emphysema. *Am J Gastroenterol.* 1968;**50**(4):289–96.

33. Caselli M, Zaffoni E, Ruina M, Sartori S, Trevisani L, Ciaccia A, et al. Helicobacter pylori and chronic bronchitis. *Scand J Gastroenterol.* 1999;**34**(8):828–30.

34. 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**(1):32–40.

35. Roussos A, Tsimpoukas F, Anastasakou E, Alepopoulou D, Paizis I, Philippou N. Helicobacter pylori seroprevalence in patients with chronic bronchitis. *J Gastroenterol.* 2002;**37**(5):332–5.

36. Kanbay M, Gur G, Akcay S, Yilmaz U. Helicobacter pylori seroprevalence in patients with chronic bronchitis. *Respir Med.* 2005;**99**(10):1213–6.

37. 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):721–4.

38. Fullerton 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):419–26.

39. Minov J, Karadzinska Bislimovska J, Vasilevska K, Risteska Kuc S, Stoleski S, Mijakoski D. Helicobacter Pyloriinfection in never-smoking male patients with chronic obstructive pulmonary disease and its relation to lung function. *Am J Immunol.* 2012;**8**(4):146–53.

40. Siva R, Birring SS, Berry M, Rowbottom A, Pavord ID. Peptic ulceration, Helicobacter pylori seropositivity and chronic obstructive pulmonary disease. *Respirology.* 2013;**18**(4):728–31.

41. Shams-Hosseini NO, Mousavi SA, Kadivar M, Ahmadipour E, Yazdani R, Moradians V. Helicobacter pylori in patients suffering from pulmonary disease. *Tanaffos.* 2011;**10**(1):31–6.

42. Ilvan A, Ozturkeri H, Capraz F, Cermik H, Kunter E. Investigation of Helicobacter pylori in bronchoscopic lung specimens of young male patients with bronchiectasis but without gastrointestinal symptoms. *Clin Microbiol Infect.* 2004;**10**(3):257–60.

43. Gulhan M, Ozyilmaz E, Tarhan G, Demirag F, Capan N, Erturk A, et al. Helicobacter pylori in bronchiectasis: a polymerase chain reaction assay in bronchoalveolar lavage fluid and bronchiectatic lung tissue. *Arch Med Res.* 2007;**38**(3):317–21.

44. Malekzadeh R, Sotoudeh M, Derakhshan MH, Mikaeli J, Yazdanbod A, Merat S, et al. Prevalence of gastric precancerous lesions in Ardabil, a high incidence province for gastric adenocarcinoma in the northwest of Iran. *J Clin Pathol.* 2004;**57**(1):37–42.