Seroprevalence of *Helicobacter Pylori* in COPD Patients in Kashmir, India

Ulfat Ara Wani[1], Umeek Jeelani[2], Shah Amir Abbas[3], Shabir Khanday[4], Hina Jeelani[5], Basharat Ara Wani[5]

ABSTRACT

Introduction: Worldwide *Helicobacter pylori* infection is the most common chronic bacterial infection of humans. The aim of the study was to evaluate the seroprevalence of *helicobacter pylori* in COPD patients in Kashmir, India

Material and methods: 50 patients with symptoms of COPD were included in the study and compared with 50 healthy controls. Blood samples (4-5 ml) were collected from patients and controls. Serum was separated and stored at -20 ºC till tested. *H. pylori* specific IgG antibodies were detected. The test procedure was carried out as per manufacturer's instructions.

Results: Mean concentration IgG ELISA anti *H. pylori* was (57.8±5.4 U/ml) in COPD patients and (37.6±4.3U/ml) in controls. There was a significant difference in concentration of anti *H.pylori* IgG ELISA between COPD patients and controls (p=0.004). There was also a significant difference in seroprevalence of *Helicobacter pylori* between COPD patients and controls (p=0.014). There was also a significant difference in High +ve IgG ELISA anti *H.pylori* seroprevalence between COPD patients with that of controls (p=0.027)

Conclusion: We concluded that the Seroprevalence of *H.pylori* is higher in COPD patients as compared to healthy controls. Also the mean concentration of anti *H.pylori* IgG is higher in COPD patients as compared to healthy controls. And the occurrence of High positive anti *H.pylori* IgG levels is higher in COPD patients as compared to healthy controls.

Keywords: Seroprevalence, *Helicobacter Pylori*, COPD

INTRODUCTION

Helicobacter pylori is a slow growing, microaerophilic spiral shaped gram negative bacteria, whose most striking biochemical characteristic is abundant production of urease. It was successfully cultured from gastric biopsy specimen from patients with histological gastritis in Perth, Australia, in 1982 and was soon named Campylobacter pylori (a name latter changed to *Helicobacter pylori*.[1]

The prevalence of chronic obstructive pulmonary disease in patients with peptic ulcer is 2-3 times greater than in ulcer free controls.[2,3] Based on these facts, many recent studies have focused on the potential association between *H.pylori* infection and various respiratory disorders.

Epidemologically, *H.pylori* infection is associated with myriad extra gastrointestinal pathologies including cardiovascular, skin, rheumatic and liver disease as suggested by various studies.[4,5]

H.pylori infection often triggers a marked local inflammatory response and a chronic systemic immune response. One hypothesis is that the persistent inflammatory response related to *H.pylori* infection could induce vascular disorders through an immune mediated release of substances associated with vasospasm or platelet aggregation.

Detection of serum anti *H.pylori* Cag A is currently the most practical investigation for predicting bacterial virulence and disease development in *H.pylori* infection.[5] Various studies has shown that these cytokines may be expressed in chronic bronchitis or its acute exacerbation.[6-9] Moreover a cross mimicry between bacterial and host antigens exists in *H.pylori* infected patients.[10] In Chronic bronchitis[8,9] and bronchiectasis[11] and other respiratory diseases the symptoms are actually an interplay of chronic inflammation and exaggerated immune response. Recently *H.pylori* has been identified in tracheobronchial aspirates in mechanically ventilated patients and possibility that it might cause ventilator associated pneumonia has been raised,[12] it appears that *H.pylori* has a close relationship with respiratory diseases.[13]

Study aimed to determine the seroprevalence of *helicobacter pylori* in COPD patients and to find out the association of *helicobacter pylori* with COPD.

MATERIAL AND METHODS

Study group: This study was conducted at the Sher– I–Kashmir institute of medical sciences, Srinagar, Jammu and Kashmir. The following groups of patients/subjects were included in the study after taking written informed consent and ethical clearance.

Group I (n=50) – Patients with COPD.

Inclusion Criteria

Chronic obstructive pulmonary disease (COPD) is a chronic disorder characterized by not fully reversible and usually progressive airflow limitation. This limitation is thought to be associated with an abnormal inflammatory response of the lungs to noxious particles and/or gases.[14]

- The airflow limitation that is not fully reversible is confirmed by spirometry (post bronchodilator FEV1 <80%) of the predicted value, in combination with an FEV1/FVC<70%.

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How to cite this article: Ulfat Ara Wani, Umeek Jeelani, Shah Amir Abbas, Shabir Khanday, Hina Jeelani, Basharat Ara Wani. Seroprevalence of *Helicobacter Pylori* in COPD patients in Kashmir, India. International Journal of Contemporary Medical Research 2016;3(5):1474-1477.

Exclusion Criteria

Following patients were excluded:

- Previous month exacerbation of COPD (the exacerbation was defined as "increased dyspnea associated with change in quality or quantity of sputum, which led the patient to seek medical attention).[15]
- Prior *H.pylori* eradication therapy.
- Anti-acid drugs or antibiotics consumption in the preceding 6 months.
- A known history of gastro intestinal tract pathology.
- A known history of vagotomy or operation on the upper gastro intestinal tract.

Group II (n=50) – consisted of Healthy Controls. Healthy controls with following criteria's were also included.

Exclusion Criteria

- A known history of chronic bronchitis
- A known history of gastro intestinal tract pathology.
- Detailed history was recorded and physical examination was performed on the patients. Routine investigations like TLC, DLC were done.

Antibody Detection

Blood samples (4-5 ml) were collected from patients and controls. Serum was separated and stored at -20 ºC till tested. *H. pylori* specific IgG antibodies were detected by using kit obtained from Adaltis Italy. The test procedure was carried out as per manufacturer's instructions.

Calculation of results

The results were calculated by subtracting the blank OD from all the reading to have the net OD values. This was followed by derivation of mean and S.D (standard deviation) of the observed OD values. The obtained value of SD was doubled to which the already obtained value of mean was added which was our cut off value. The ratio between the average net O.D value of sample and that of cut off was calculated. This ratio is the cut off index (COI). The sample is considered positive if ratio is > 1.2, negative if the ratio is <0.8, border line if between 0.8 to 1.2 (cut off±20%). If the result was doubtful the test was repeated.

STATISTICAL ANALYSIS

Statistical analysis was performed by using statistical package for social sciences (SPSS software version 13, Chicago IL). A p-value of < 0.05 was taken significant

RESULTS

A total of 100 subjects were selected which comprised of 50 COPD patients and 50 controls. Maximum number of COPD patients were in age group 51-60 years (40%) and controls in age group of 41-50 years (42%). For COPD patients male to female ratio and the rural to urban ratio were [(1.17:1) and (1.38:1)] and for controls the ratio's were [(1.38:1) and (2.125:1)] respectively. There was significant difference in rural to urban ratio between COPD patients and controls (p=0.052). Majority of COPD patients (76%) and controls (62%) were current smokers. There was a significant difference in smoking status (current smokers versus non-smokers plus ex-smokers) between COPD patients and controls (p=<0.001)

Clinically patients presented with cough with expectoration or cough with expectoration and breathlessness while controls were asymptomatic. PFT of the COPD subjects showed that 32 COPD patients (64%) were in GOLD Stage I and 18 COPD patients (36%) were in GOLD Stage II with a ratio of (1.77:1), while general investigation profile of the studied subjects showed that 5 COPD patients presented with anemia (10%) and none among the controls.

Mean concentration IgG ELISA anti *H. pylori* was (57.8±5.4 U/ml) in COPD patients and (37.6±4.3U/ml) in controls. There was a significant difference in concentration of anti *H.pylori* IgG ELISA between COPD patients and controls (p=0.004) (Table-1).

There was a significant difference in seroprevalence of *Helicobacter pylori* between COPD patients and controls (p=0.014). There was also a significant difference in High +ve IgG ELISA anti *H.pylori* seroprevalence between COPD patients with that of controls (p=0.027) (Table-2).

DISCUSSION

Recent studies suggest an epidemiological association between *helicobacter pylori* infection and several extra gastro duodenal pathologies, including cardiovascular, rheumatic, skin and liver diseases.[4,5,10] In Chronic bronchitis[8,9] and bronchiectasis[10,11] and other respiratory

IgG ELISA for Anti H Pylori	min	max	Mean	SE	Inter group Comparison	p value
COPD	1	100	57.8	5.4		
Control	1	100	37.6	4.3	G1 and G2	0.004

Table-1: IgG ELISA concentration for anti H Pylori in the studied subjects

Sero-prevalence		COPD		Control		Total		p value
		n	%	N	%	n	%	
Sero-prevalence for H-Pylori	Positive	39	78.0	25	50.0	99	67.3	a: p=0.684, b: p=0.014, c: p=0.004, d: p=0.005
	Negative	11	22.0	25	50.0	48	32.7	
Result	+Ve	16	32.0	18	36.0	56	38.1	a: p=0.085, b: p=0.994, c: p=0.027, d: p=0.071
	-Ve	11	22.0	25	50.0	48	32.7	
	High +Ve	18	36.0	6	12.0	33	22.4	
	Boder line	5	10.0	1	2.0	10	6.8	

Table-2: Sero-prevalence of H Pylori as per IgG ELISA of the studied subjects

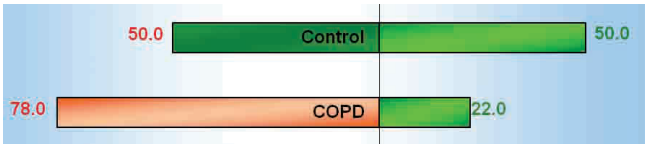
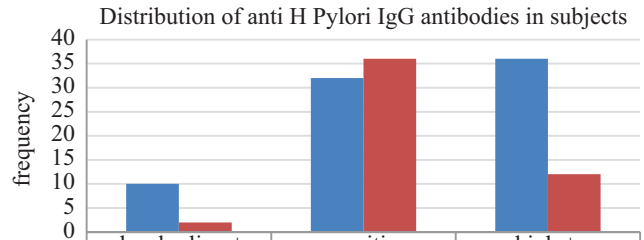


Figure-1: Seroprevelance of H Pylori as per IgG ELISA in studied subjects



	border line +ve	positive	high +ve
COPD	10	32	36
Control	2	36	12

Figure-2: H Pylori as per IgG levels in studied subjects

diseases the symptoms are actually an interplay of chronic inflammation and exaggerated immune response with evidence of *H.pylori* infection evidence by seropositivity.[16] However, such association has not been seen in bronchial asthma suggesting that *H.pylori* may have a role in COPD especially when smoking history is lacking. At present, there is no definite proof of a causal relationship between H pylori and respiratory diseases, since most studies are case- control studies, with small sample size.

The pathogenisis or the association between the two can be due to inflammatory mediator activation. So measuring the concentration of these mediators, virulence factors based on various starins and genetic predisposibility of the infected host in *H.pylori*-infected patients with respiratory diseases need further evaluation.

In the present study, a high seroprevalence in patients with COPD as compared to control subjects was observed (p=0.005). The difference in seroprevalence also exists when COPD patients are compared with control subjects as different groups (p value:0.004 and 0.035 respectively). The inference being simple that possibly in *H.pylori* infection there is triggering of a cascade of inflammation to produce the disease. These observations are also recorded by many studies conducted even with larger sample sizes than our.[16-19] Our results are consistent with study conducted by Kanbay Mehmet[20] in which 66% chronic bronchitis patients versus 57.7% controls tested positive for *H.pylori* (p=0.008). In study conducted by Roussas- et-al in Greece[17] 83.3% chronic bronchitis where anti *H.pylori* IgG positive (p=0.007) as compared to 60% healthy controls. However the sample size of study was large as compared to our study. In Dr Anastasios Roussa's study[19] the prevalence of *H.pylori* in patients and controls was 77.8% and 54.7% respectively (p<0.001). A Chinese study[18] showed that there was no significant difference between chronic bronchitis and peptic ulcer group for anti *H.pylori* IgG (p is more than 0.05). However these serological parameters were significantly higher in patients with chronic bronchitis or peptic ulcer than in control groups (p<0.01)

When we compared seroprevalence as number of high positive, positive, borderline and negative in different groups a statistically significant high positive results were observed in COPD group compared to control group (p=0.001).This stronger association between COPD and *H.pylori* needs to be confirmed as unfortunately none of the studies conducted studied this parameter. One can postulate that a higher burden of infection to produce substantial amounts of pro-inflammatory markers is possibly needed to produce COPD. However, it is too early to conclude as our sample size and others too, is not large enough to draw conclusions. However, the main outcome of the study prevailed as we found a significant difference in antibodies against *H.pylori* amongst COPD patients as compare to control group (p=0.011). nearly similar study results were found by Dr. Anastasios Roussas from SOTRIA Chest Diseases Hospital in Athen.[17] However, the absolute values don't match between our study and his, due to difference in ELISA kit standardizations as per manufacturers protocol.

The reported association between chronic bronchitis and development of ulcerogenesis was attributed to cigratte smoking in previous studies[21] where smoking was regarded as independent factor, however the relationship between cigarette smoking and *H.pylori* is controversial. The prevalence of H.pyolri infection in smokers has been reported as low,[22] normal,[23] or high.[24]

The influence of smoking on the prevalence of chronic bronchitis has also been little understood.[25] Therefore we tried to compare the current smoking status of COPD patients and controls (also smokers and never smokers) and found no statistical difference. Hence an inference can be made that smoking does not predispose to *H.pylori* infection as seroprevalence significantly differed between COPD and control groups. Similar observations have been made by the chinese study.[18]

Hence we postulate that chronic activation of inflammatory mediators induced by *H.pylori* infection might lead to development of COPD. It is well known that *H.pylori* stimulate the release of a variety of pro-inflammatory cytokines, including IL-1,IL-8 and TNF-α.[26,27] Moreover, eradication of *H.pylori* leads to normalization of serum cytokines level.[28] These cytokines are also thought to be involved in the pathogenesis of chronic bronchitis.[7,8,11] Therefore, *H.pylori* function in general and Cag A positive strains in particular might play a pro-inflammatory role in co-triggering chronic bronchitis with other more specific environmental genetic and some unknown factors.

CONCLUSION

We concluded that the Seroprevalence of *H.pylori* is higher in COPD patients as compared to healthy controls. Also the mean concentration of anti *H.pylori* IgG is higher in COPD patients as compared to healthy controls. And the occurrence of High positive anti *H.pylori* IgG levels is higher in COPD patients as compared to healthy controls.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 26-03-2016; **Published online**: 30-04-2016