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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**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

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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 gastritis and peptic ulcer diseases[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
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 questionnaire 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 Medizinische 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 Medizinische 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 obstructive 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%)    | *P*<0.912          | 17/21 (81.0%)      | *P*<0.522          |
| Female      | 10/10 (100.0%)   |                    | 7/11 (63.6%)       |                    |
| Total       | 24/27 (88.9%)    |                    | 18/21 (85.7%)      |                    |
| Age group   |                  |                    |                    |                    |
| 20–40       | 11/11 (100.0%)   |                    | 8/10 (80.0%)       |                    |
| 41–60       | 8/10 (80.0%)     |                    | 7/10 (70.0%)       |                    |
| Above 61    | 5/5 (83.3%)      |                    | 6/6 (100.0%)       |                    |
| Nutritional Status (BMI) | |                   |                    |                    |
| Normal      | 9/10 (90.0%)     |                    | 6/10 (60.0%)       |                    |
| Overweight  | 2/2 (100.0%)     |                    | 1/1 (100.0%)       |                    |
| Underweight | 1/1 (100.0%)     |                    | 0/0 (00.0%)        |                    |
| Socioeconomic Status |         |                    |                    |                    |
| Low income  | 9/9 (100.0%)     |                    | 6/6 (100.0%)       |                    |
| Middle income | 7/7 (100.0%)   |                    | 5/5 (100.0%)       |                    |
| High income | 8/8 (100.0%)     |                    | 7/7 (100.0%)       |                    |

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 obstructive airway diseases to control by Western blot.

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

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 (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 infection and asthma. Thus analysis of the results pertaining to demography (Table 2) revealed that 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

| Western blot antigen H. pylori | IgG-anti H. pylori in COPD seropositivity (%) | IgG-anti H. pylori control seropositivity (%) | IgG-anti H. pylori asthma seropositivity (%) |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| P20, cagA                   | 15/20 (75.0)               | 15/20 (75.0)               |                             |
| P95, vacA                   | 12/21 (57.1)               | 12/20 (60.0)               | 12/20 (60.0)               |
| p75                         | 0/21 (0.0)                 | 1/20 (5.0)                 | 1/20 (5.0)                 |
| P67, Flag                   | 13/21 (61.9)               | 14/20 (70.0)               | 11/20 (55.0)               |
| P66, ureB                   | 18/21 (85.7)               | 15/20 (75.0)               | 14/20 (70.0)               |
| P57                         | 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 IFA 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 IFA 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 IFA 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 IFA investigations revealed a positive correlation between H. pylori infection and obstructive airway diseases (COPD and asthma).

Table 4

| Demographic | COPD vs. control | Significance value | Asthma vs. control | Significance value |
|-------------|-----------------|--------------------|--------------------|--------------------|
| Gender      | Male            | 14/17 (82.4\%)     | 4/10 (40.0\%)      | P<0.045            |
| Female      | 2/21 (9.5\%)    | 2/21 (9.5\%)       | 2/21 (9.5\%)       | P<0.045            |
| Total       | 16/38 (42.1\%) | 6/20 (30.0\%)      | 4/21 (19.0\%)      | P<0.012            |
| Age group   | 20–40           | 6/11 (54.5\%)      | 7/20 (35.0\%)      | P<0.014            |
| 41–60       | 3/11 (27.3\%)   | 4/10 (40.0\%)      | 5/10 (50.0\%)      | P<0.007            |
| Above 61    | 5/8 (62.5\%)    | 0/5 (0.0\%)        | 0/5 (0.0\%)        | NA                 |
| Nutritional Status | Normal | 7/10 (70.0\%) | 3/12 (25.0\%) | P<0.000            |
|              | Overweight      | 1/1 (100.0\%)      | 0/3 (0.0\%)        | NA                 |
|              | Underweight     | 1/1 (100.0\%)      | 0/3 (0.0\%)        | NA                 |
| Socioeconomic Status | Low income | 5/8 (62.5\%) | 2/10 (20.0\%) | P<0.000            |
|              | Middle income   | 4/8 (50.0\%)       | 2/13 (15.4\%)      | P<0.061            |
|              | High income     | 1/2 (50.0\%)       | 1/2 (50.0\%)       | P<0.034            |

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 IFA. The IFA 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.

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