Evaluation of Putative Association Between Celiac Disease and *Helicobacter pylori* Infection in Hyderabad, Sindh

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Abstract. Celiac disease is an autoimmune mediated enteropathy caused by ingestion of gluten and affects approximately 1% of the population. It has significant deleterious effects on health and mortality. *Helicobacter pylori* is the major causative agent of peptic ulcer and gastric cancer. The aim of this study was to assess the prevalence of both *H. pylori* (HP) and celiac disease (CD) as mono and co-infections and to find out the possible association between CD and presence of HP. A total of 172 blood samples from individuals suspected of CD and HP infection were collected during March to December, 2015 from Hyderabad. Serum analyzed for presence of anti-HP antibodies for evaluation of HP infection and tissue transglutaminase IgA antibodies (tTGA) for detection of CD. The serodiagnosis data revealed that out of 172 samples tested 19.19% were positive for CD which included 6.98% (n=12) male patients, whereas 12.21% (n=21) were females. The HP was detected in 44.77% (n=77) including 22.09% (n=38) males and 22.67% (n=39) females out of the 33 CD positive cases, HP was detected in 17 (9.88%). HP in CD positive cases was found to be 6.60% in males 9.43% in females respectively. The HP prevalence in non-CD patients appeared to be high such as 18.02% and 16.86%. In conclusion, the data of the present study indicates that HP is more prevalent than CD.

Keywords: celiac disease, *Helicobactor pylori*, prevalence, peptic ulcer

Introduction

Celiac disease (CD) is an autoimmune disorder triggered by ingestion of gluten protein and its ilks and affects the small intestine in approximately 1% of population. It causes very serious effects on health and may leads to mortality (Corrao *et al*., 2001; Logan *et al*., 1989). When persons affected with CD, consume the gluten present in rye, wheat, and barley, their immune system destroys the micro-villi in the small intestine. Micro-villi play very active role is absorption of nutrients from small intestine. They increase the surface area, thus maximum of the nutrients can be absorbed by them. However, persons with damaged microvilli are unable to absorb nutrients as the surface area is not as large as in healthy persons, which may leads to the deficiency of vitamins and other nutrients into the body due to poor absorabance capacity of damaged villi (Fasano, 2009). Only way to treat CD is exclusion of gluten from diet. In this way, it has been reported that the damaged micro-villi start repairing themselves and may fully recover within 1-2 years. CD occurs in genetically susceptible persons due to consumption of gluten

containing grains of wheat rye and barley (Fasano, 2009).

Over the past two decades, however, scientists have been able to know very-well and enhanced their understanding of CD. Its now a well-known fact that CD is a an immune mediated enteropathy, resulting due to the attack of the immune system on the individul's own body's tissues (Fasano *et al*., 2003). Thus, the disease can be casued due to a combination of factors such as gluten intake, abnormalities in small intestine and due to various predisposing genes. A wide range of symptoms have been reported to menifest during the course of disease. Some of the well known symptoms of CD include chronic diarrhea, vomiting, abdominal distension, weight loss and fatigue (or stunted growth in children) (Fasano, 2009).

In general, there is considerable variation in the prevalence rate of HP based on the geographical locations (Tunio *et al*., 2018). Geographically, the prevalence of HP has been described to be associated with the living standards during childhood and inversely correlates with socio-economic status of the individuals (Malaty and Graham, 1994). In western countries, prevalence of HP has been reported higher in immigrants from the

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developing world (Perez-Perez *et al*., 2005). In developing countries the bacterium affects the children during their first five years of age, indicating that HP is acquired during early childhood (Fiedorek *et al*., 1991). On the contrary, owing to improved hygiene and sanitation in industrialized world, the infection rate in early stage of life appears to be at a very low rate and then increases with the increase in age. Elimination of HP can be achieved by improved hygiene and the infection rate children may be lowered by improving the housing conditions (Rehnberg-Laiho *et al*., 2001; Roosendaal *et al*., 1997). In general, new incidences of HP infection occur in childhood, if it is not treated properly. The aim of presnt study was to detect anti-HP antibodies and prevalence of CD using anti- tTGA (tissue transglutaminase IgA) antibodies using ELISA and to find out the association between CD and HP infection.

Materials and Methods

Sampling population, study duration and location. The target population for this prospective study included patients referred by doctors of gastrointestinal clinics of Hyderabad. Research work was carried out at Healthcare Molecular and Diagnostic Laboratory and Institute of Microbiology. A total of 172 blood samples from patients suspected of either CD or HP, were collected and examined over a period of eight months during March to December, 2015 from Hyderabad.

Blood collection and serum separation. A 5 mL blood sample was taken from each individual and immediately added to gel tubes for serum separation. Serum was separated by centrifugation at 4000 rpm for 5 min. Serum was either stored at -20°C or used immediately in ELISA assay for assessment of antibodies against CD and HP. The samples were assessed for presence of antibodies against CD and HP by using an enzyme immunoassay.

Diagnosis of CD using serum anti- tTGA IgA antibodies. The diagnosis of CD was performed by assessing the presence of anti-tTGA using an enzyme immunoassay as per manufacturer's instructions. Briefly, 5 mL blood sample was collected from all the patients enrolled in this study. Serum was separated using gel tubes followed by centrifugation at 3000 rpm for approx. 10 min. Grossly hemolysed blood was excluded, anti-tissue transglutaminase (TTG) IgA were assessed to detect CD using ELISA technique using commercially available transglutaminase IgA ELISA Kit (Biomerica,

Germany). The test positivity was considered on the basis of cutoff level, if the cutoff level was = 5 units/mL the test was recorded positive, as recommended by the manufacturer. Individuals were regarded positive for CD, when tTGA antibodies were above the cutoff level (transglutaminase IgA ELISA Kit [Biomerica, Germany]).

Diagnosis of HP infection using serum anti- HP IgG antibodies. The GAP IgG kit is a quantitative ELISA assay used to detect antibodies from human serum against HP. Microtiter plate wells are coated with partially purified HP antigens and patient's serum is added to each of the wells. Antibodies specific to HP if present in the serum will bind to antigen in the wells. Subsequently, anti-human IgG enzyme conjugate was added to each of the wells including blanks and allowed to react with antigen-antibody complex, which produces color. Diagnosis of HP was done using anti-HP antibodies in an enzyme immunoassay. Briefly, 5 mL blood was collected, and serum obtained was used for detection of anti-HP antibodies using GAP-IgG ELISA Kit (Biomerica, Germany). A cutoff level of = 5 units/mL was set for positive samples as suggested by the manufacturer. The presence of anti-HP antibodies above the cutoff level described by the manufacturer, the subject was considered as HP positive patient (transglutaminase IgA ELISA Kit [Biomerica, Germany]). The data was analysed in Microsoft Excel and due to nominal data it was analyzed as number and percentage.

Results and Discussion

Over all prevalence of HP and CD. Out of 172 patients, 63.95% were "test positive" for both CD and HP. Male patients were 47.67% (*n*=82) and female 52.33% (*n*=90). Of these, 44.77% (*n*=77) patients yielded positive results for presence of HP IgG antibodies. Sero-diagnosis of CD demonstrated that 19.19% were test positive for tTGA antibodies thus were considered CD positive. Dual infection was screed into all samples and only 9.88% (*n*=17) patients yielded positive serology test for both CD and HP dual infection (Fig. 1).

Gender-wise distribution of CD and HP. This is a first study comparing the prevalence of HP in CD patients in Hyderabad. A total of 172 samples were taken from the patients referred for diagnosis of CD and HP and analyzed using ELISA. The presence of the anti tTGA antibodies indicated the CD disease whereas anti-HP antibodies were used for detection of
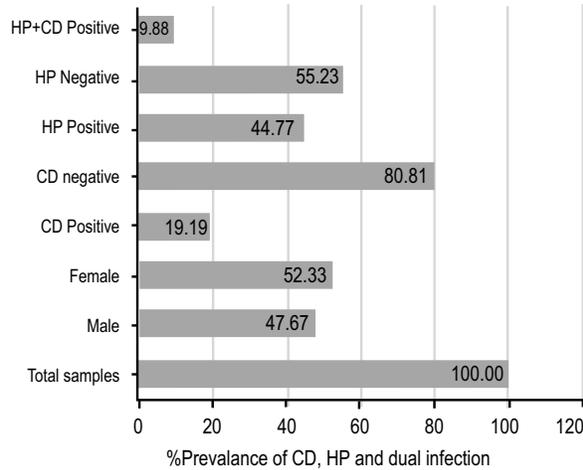
Fig. 1. Over all prevalence of CD and HP.

HP. The sero-diagnosis data demonstrated that out of 172 samples tested 19.19% were positive for CD which included 6.98% (*n*=12) male patients whereas 12.21% (*n*=21) were females. Females were found higher in number than males affected with CD. Since the tTGA

assay is highly specific and sensitive, the patients yielding positive results may be affected by enterosopic enteritis and if untreated may develop severe enteropathy in future. The HP was detected in 44.77% (*n*=77) including 22.09% (*n*=38) male patients and 22.67% (*n*=39) females. HP infection was equally distributed in both female and male patients. Prevalence of HP was higher than CD. Comprehension of CD and HP positivity in different age groups (10 years) is shown in Table 1 and 2. There were difference in the prevalence between both genders for both CD and HP infections; however, CD appeared more prevalent in females than males.

Prevalence of dual infection by HP and CD. Out of 172 patients 9.88% (*n*=17) were positive for both CD and HP infections thus were considered as dual infection patients (Fig. 2). Among them 4.07% (*n*=07) were male and 5.81% (*n*=10) were females (Fig. 2).
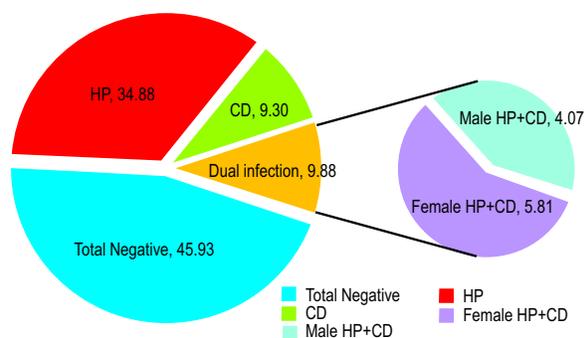
Comparative study of HP and CD in male and female subjects. The comprehension of the prevalence rate of HP and CD by gender and age-wise has been shown in Fig. 3 and 4. HP incidences were found to increase

**Table 1.** Age-wise distribution of male and female CD patients

Age	Male				Female			
	Total samples	Anti-TTG +ve		Anti-TTG –ve	Total samples	Anti-TTG +ve		Anti-TTG–ve
	(=*n*)	(=*n*)	(%)	(=*n*)	(=*n*)	(=*n*)	%	(=*n*)
0 - 10	9	3	1.74	6	12	5	2.91	7
11 - 20	14	3	1.74	11	15	5	2.91	10
21 - 30	19	2	1.16	17	17	4	2.33	13
31 - 40	13	1	0.58	12	18	3	1.74	15
41 - 50	9	1	0.58	8	14	2	1.16	12
51 - 60	9	1	0.58	8	5	1	0.58	4
61 - Above	9	1	0.58	8	9	1	0.58	8
Total	82	12	6.98	70	90	21	12.21	69

Table 2. Age-wise distribution of male and female HP patients

Age	Male				Female			
	Total samples	Anti-HP +ve		Anti-HP –ve	Total samples	Anti-HP +ve		Anti-HP –ve
	(=*n*)	(=*n*)	(%)	(=*n*)	(=*n*)	(=*n*)	%	(=*n*)
0 - 10	9	1	0.58	8	12	1	0.58	0
11 - 20	14	5	2.91	9	15	4	2.33	5
21 - 30	19	7	4.07	12	17	7	4.07	7
31 - 40	13	7	4.07	6	18	7	4.07	5
41 - 50	9	6	3.49	3	14	7	4.07	3
51 - 60	9	6	3.49	3	5	6	3.49	1
61 - Above	9	6	3.49	3	9	7	4.07	1
Total	82	38	22.09	44	90	39	22.67	22

Fig. 2. Frequency of dual CD and HP infection in both genders.
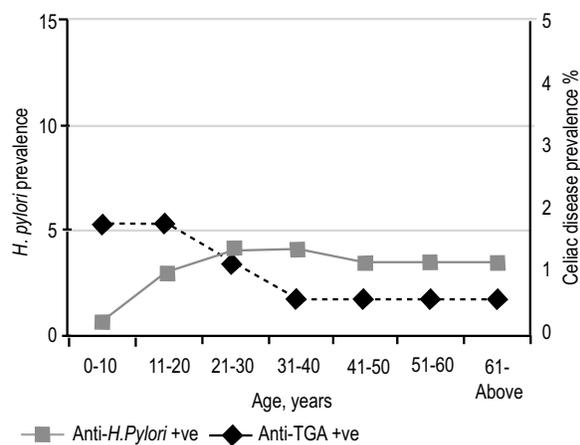
Fig. 3. Comparative analysis of age-wise prevalence of HP and CD in male.
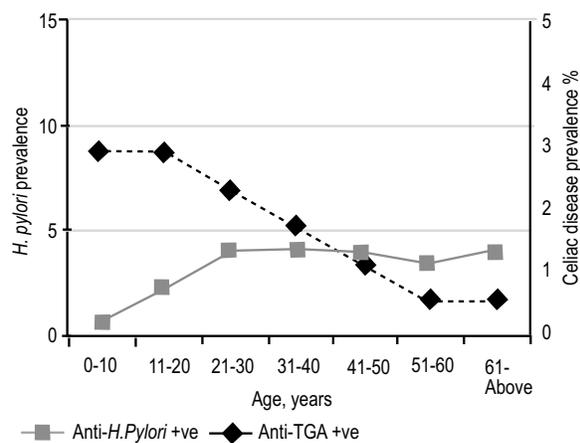
Fig. 4. Comparative analysis of age-wise prevalence of HP and CD in female.

with increase in age, as the prevalence of HP was seen highest in old age patients, while the CD was found to be more prevalent in the younger age groups as compared with the older age individuals. The findings of the present study demonstrate that the relationship between HP and CD was similar in all age groups. The relationship between men and women was almost similar without substantial difference.

Gender-wise correlation between CD and HP. The findings of the present study revealed that that the prevalence of HP was significantly higher than CD. However, HP was considerably less common in CD patients as compared with the non CD patients. Out of the 33 individuals who showed positive serological test for CD, HP was detected in 17, which is 9.88% of all patients tested for both diseases. HP in CD positive cases was found to be 6.60% in males and 9.43% in females respectively (Table 3). Whilst the HP prevalence in non-CD patients appeared to be high 18.02% and 16.86% suggesting as inverse correlation between CD and HP. The findings of present study indicate an inverse relationship of CD with HP.

The present research study assesses the correlation between CD and HP infection in patients referred to gastrointestinal clinics in Hyderabad, Sindh. The prevalence of CD has been reported to increase worldwide due to reasons not known yet (Lohi *et al.*, 2007). However, different environmental factors have been suggested in various research studies. As per hygiene hypothesis, autoimmune may trigger as a consequence of decreased exposure to bacterial antigens (Lebwohl *et al.*, 2013). Hence, considering the importance of the exposure to micro-organisms in decreasing the risk of acquiring CD, the present study aimed to investigate the association between CD and HP. CD is an autoimmune mediated enteropathy that develops due to ingestion of gluten. In the present study, the patients having symptoms of CD and/or HP were enrolled. Majority of the patients belonged to females

Table 3. Association between CD and HP

Characteristics	no. of CD	HP prevalence in CD		HP prevalence in non-CD	
Gender	(=*n*)	(=*n*)	(%)	(=*n*)	(%)
Male	12	7	6.60	31	18.02
Female	21	10	9.43	29	16.86
Total	33	17	9.88	60	34.88

as compared to males. Incidences of seropositivity were higher for HP infection. It was also observed that younger individuals were less positive for HP than the older individuals, in both genders. Serodiagnosis of CD demonstrated that 19.19% were CD positive. The genderwise distribution of CD data revealed that females were considerably higher in number than males affected with CD. Due to the fact that anti tTGA assay is a highly sensitive and yields high positivity, the patients positive for tTGA more likely have enterosopic enteritis (Verma *et al*., 2018; DeGeorge *et al*., 2017). CD positive cases were lower than that of HP. Females were more susceptible than males for CD. Unlike HP, CD negative patients were older that CD negative subjects in both genders.

The HP was detected in 44.77% sample and it was equally distributed in both male and female patients. Prevalence of HP was higher than CD. HP incidences were found to increase with increase in age, as the prevalence of HP was seen highest in old age patients, while the CD prevalence was found higher in the young age groups strata. CD and HP were more common in females than males. Gender-wise analysis of data revealed that the prevalence of HP was more common in men (Sonnenberg *et al*., 2010), whilst CD was observed to target more to the women (Lebwohl *et al*., 2012). HP prevalence has been shown to increase with age (Sonnenberg *et al*., 2010), and socio-economic status wise HP has often been described to be common among the individuals with lower socio-economic status (Grad *et al*., 2012; Sonnenberg *et al*., 2010), whereas CD unlike HP infection may be less common among the individuals with lower socio-economic status (Olén *et al*., 2012). Dual infection was screened into all samples and only 9.88% patients yielded positive serology test for both CD and HP antibodies, thus were considered as dual infection patients. The rate of HP prevalence was found less in patients suffering from CD as compared with the patients without CD. Whilst, the HP incidences in non-CD individuals appeared to be high suggesting that the presence of HP may have immunomodulatory effects in against developing CD. Moreover, some limitations of this study could be the detection of CD only by tTGA serum antibodies as there are more tests to confirm the CD. HP was also detected by serological methods, while the gold standard is duodenal biopsy for diagnosis of both CD and HP, which was not performed in the present study due to time constraints and non-availability of funds.

Conclusion

In conclusion, the prevalence of HP has been shown to occur during early years of life, CD may affect at any stage of life. It is, therefore considered that the presence of HP is early stage may likely to protect against the development of CD by modulating the immune response by unknown mechanism. Investigations regarding the relationship between CD and HP infection have yielded contradictory results, probably because of various factors including the different prevalence rates, samples sizes used in this study and the techniques used for screening of both diseases.

Conflict of Interest. The authors declare no conflict of interest.

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