**Editorial**
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*J Immunol Clin Microbiol*

# The two major branches of medicine and an innovative journal

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Welcome to Journal of Immunology and Clinical Microbiology (JICM): a new journal of medical sciences.

With this paper we are very pleased to introduce a new innovative journal (Thanks to our friend Dr. Erkan Yula for his admirable effort). The *JICM* is publishing refereed, original papers in a broad range and we hope it will prove useful to everyone in all areas of clinical microbiology and immunology; it is planned to be particularly valuable for trainees, early and late career researchers in immunology and medical microbiology.

The intention of *JICM* will be publishing various types of articles covering all areas of immunology, allergy, autoimmunity, rheumatology, pharmacology, immunopharmacology, immunotherapy and vaccines, cancer immunology, transplantation immunology, infectious diseases, clinical microbiology, bacteriology, virology, parasitology, mycology.

We would like to publish your academic studies in both written and visual forms. The article types to be considered are as follows; original article (research article), review, short/mini review, case series, technical note, letter to editor / short communication, editorial, hypothesis, highlights and focus (five things to know about), field report/national and international reports, video article and science education.

In addition, the *JICM* publishes peer-reviewed scientific "video articles" to help learning new skills related fields with immunology and clinical microbiology. The "Video article" is a new type of publishing method of scientific journals which are 10-20 minute short videos about specific topics using content from articles and other text sources.

Progress in science continues at a dazzling speed; our knowledge of species, functional composition and effects on humans of the human microbiome is rapidly increasing, but it is still based on very few cohorts and little is known about variation across the world. The various interactions that have been shown to exist between hosts and their microbial inhabitants, explanation will have required new technologies and an interdisciplinary approach.

Over the past decades, there have been numerous advances in our current understanding of the immune system and how it functions in particular to protect the body from infection. Given the complex nature of this subject, there are many unclear topics of innate and acquired immunity, immunization, immunopathologies and immune related diseases and their therapy.

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Antimicrobial resistance threatens the effective prevention and treatment of an ever increasing range of infections caused by viruses, bacteria, parasites and fungi. According to the CDC report; each year in the US, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people dying each year as a direct result of these infections (CDC, Antibiotic Resistance Threats in the United States, 2013).

Although the immunosuppressant drugs allow the transplanted organ to remain healthy and free from damage and also are used to treat autoimmune diseases, the statement by Dr. Friedman "You need to take enough of your medicines to prevent organ rejection. But you can't take so much that your risk of infection gets too high" is still valid.

*JICM* will be committed to the good publication guidelines; rapid processing and fast peer review, no publication charge, a minimum of two rounds of expert scientific editing, advantages of Open Access for research dissemination (increased readership, visibility, usage and impact of your work), authors retain copyright under a Creative Commons license and access for researchers in all countries.
Writing a research paper is a skill and young academicians need to improve. *JICM* have the team of experienced scientists and academicians, who are dedicated to help learn and improve that (your) skill by providing a guide for writing high quality research papers through peer reviewing.
We are honoured to have a diverse Editorial Board with reputable experts from Turkey and other countries. We thank the academicians involved in the editorial board. We are all ready to invest our efforts in making *JICM* a journal which will be continually improving, attractive and pleasant to read.

Don't wait! Start submitting your first paper to *JICM* now; we look forward to reading it.

I wish you all a good time in the company of our journal and hope that you will enjoy the diversity of the JICM.

**On behalf of The *JICM* Editorial Team**

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# The distribution of Rotavirus G and P genotypes in children with acute gastroenteritis in Cukurova region, Turkey

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## Abstract

**Background:** Rotavirus is the major cause of acute gastroenteritis in infants and young children worldwide. The aim of the study was to determine the frequency of rotavirus infection and the distribution of rotavirus G and P genotype combination among children under 5 years of age with acute gastroenteritis in Cukurova region, Turkey, between October 2009 and June 2010.

**Material and Methods:** The stool specimens (n=846) collected from children with acute gastroenteritis were analyzed by enzyme-linked immunosorbent assay (ELISA) for group A rotavirus antigen. Semi-nested multiplex reverse transcription-polymerase chain reaction (RT-PCR) test was performed for rotavirus G and P genotyping.

**Results:** The rate of rotavirus infection was found to be in 144 patients (17%). The predominant rotavirus genotype was G1P[8] (22.2%), followed by G1P[4] (17.3%), G2P[4] (13.8%), G9P[4] (6.3%), G9P[8] (4.8%), G2P[8] (2.8%), G1P[10] (2.1%) and G4P[8] (1.4%). The most common G genotype was G1 (41.7%), followed by G2 (16.6%), G9 (11.1%) and G4 (1.4%). Rotavirus P[4] genotype was identified in 37.5%, P[8] in 31.2% and P[10] in 2.1% of samples. The prevalence of mixed rotavirus infections was 29.2% (n=42).

**Conclusion:** Although the predominant rotavirus genotypes circulating during the study period in our region are targets of current rotavirus vaccines, uncommon, non-vaccine rotavirus genotype combinations such as G1P[4] and G9P[4], which might appear to be the result of mixed rotavirus infections with high rate (29.2%), were also detected. G1 is included in both recent rotavirus vaccines. The continuous investigation of molecular epidemiology of rotavirus infections is essential to evaluate the effectiveness of rotavirus vaccines.

**Key words:** Rotavirus, Gastroenteritis, Genotypes, ELISA, RT-PCR.

## Introduction

Rotavirus is the most common cause of acute severe gastroenteritis in infants and young children worldwide. It is estimated that approximately 600,000 children die annually due to rotavirus gastroenteritis and more than 80% of deaths occur in developing countries. In developed countries, rotaviruses are associated with high morbidity, but low mortality and are the most frequently isolated pathogens in children hospitalized with acute gastroenteritis (1).

The rotavirus belongs to the family Reoviridae, which is non-enveloped with an 11 segmented double-stranded RNA genome surrounded by a triple-layered icosahedral capsid: core, inner and outer capsid. Rotaviruses are divided into seven groups named from A to G, according to the differences in inner capsid protein VP6. Groups A, B, and C rotaviruses infect humans and animals, whereas the D-G groups infect only animals. Group A rotaviruses are the main cause of severe gastroenteritis in children worldwide (2, 3).

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The genes encoding the outer capsid viral proteins VP7 and VP4, which are responsible for cell attachment and entry, allow the classification of group A rotaviruses into G and P genotypes, respectively. Up to now, at least 27 G types and 35 P types have been described for rotavirus group A, but it has been known that only 10 G (G1-G6, G8-G10, and G12) and 11 P (P1, P3-P6, P8-P11, P14 and P19) genotypes infect humans (4). Furthermore, epidemiological studies have shown that five of the G genotypes (G1, G2, G3, G4, and G9) and three of the P genotypes (P1A[8], P1B[4], and P2A[6]) are globally common in humans (1,3,5,6). Another issue that is of critical importance is the emergence of new reassortant virus strains in case of coinfection with different rotavirus strains. There is a large number of G and P genotype combinations circulating in the human population, however five major combinations of G and P genotypes worldwide are G1P[8],G2P[4], G3P[8], G4P[8], and G9P[8] (1,5).

Considering the genetic diversity of rotaviruses, it is advisable to determine geographic variation of predominant strains for effective rotavirus vaccine development. At present, there are two rotavirus vaccines approved in many countries including Turkey, which are Rotarix (GlaxoSmithKline, Research Triangle Park, NC, USA), a live attenuated monovalent vaccine containing rotavirus P1A[8]G1 genotype and RotaTeq (Merck, Rahway, NJ, USA), a live attenuated pentavalent vaccine consisting of G1, G2, G3, G4, and P1A[8] types. However, rotavirus vaccines are not yet included in national immunization programme in Turkey. The efficacy of the Rotarix and RotaTeq against severe diarrhea was found to be 85% and 98%, respectively (3, 7). While deciding the strategy for immunization against rotavirus, it is important to detect the regional incidence of rotavirus gastroenteritis and predominant circulating genotypes before and after immunization.

The aim of this study was to determine the frequency of rotavirus infection and the distribution of rotavirus G and P genotypes among children under 5-years-old with acute gastroenteritis in Çukurova region, Turkey, between October 2009 and June 2010. The results of this study would provide epidemiological information before national implementation of rotavirus vaccines into routine childhood immunization schedule in Turkey.

## Material and methods

### *Sample collection*

A total of 846 stool specimens were collected from unvaccinated children with acute gastroenteritis, younger 5 years of age, admitted to Adana Obstetrics and Child Care Hospital from October 2009 to June 2010. The stool samples were stored at -80ºC until tested. Clinical symptoms and demographic data of the patients were obtained via a questionnaire filled out by a physician by face to face interview and after physical examination of the patients.

### *Rotavirus antigen detection*

Stool samples were tested for group A rotavirus antigen using a solid phase sandwich type enzyme immunoassay (Ridascreen rotavirus ELISA test, R-Biopharm AG, Germany). Rotavirus antigen-positive samples were evaluated for rotavirus G and P genotyping by semi-nested multiplex RT-PCR test using the primers previously described by Gentsch et al.,(8) Gouvea et al.,(9) and Iturriza-Gómara et al.(10).

### *RNA extraction from stool samples*

Frozen stool samples were incubated on the bench to dissolve and then diluted 1:10 in phosphate-buffered saline. Viral RNA was extracted using the High Pure Viral RNA Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instructions.

### *RT-PCR for rotavirus G and P typing*

The G and P genotyping of rotavirus was performed by using two-step RT-PCR. For rotavirus G genotyping, the 1062 bp fragment of the VP7 gene was amplified with the forward primer Beg9 (5'- GGC TTT AAA AGA GAG AAT TTC CGT CTG G- 3') and the reverse primer End9 (5'-GGT CAC ATC ATA CAA TTC TAA TCT AAG-3'). For P genotyping, the consensus primers Con2 (5'-ATT TCG GAC CAT TTA TAA CC-3') and Con3 (5'- TGG CTT CGC CAT TTT ATA GAC A-3') were used to amplify *VP4* gene fragment of the 876 bp (8-10). For G genotyping, the reaction was carried out with an initial reverse transcription step. Synthesis of cDNA was carried out in 20µl reaction volume by using 10U of reverse transcriptase enzyme (Roche Diagnostics, Mannheim, Germany), 25 pmol of each Beg9, and End9 primers, 20U RNasin (Roche, Diagnostics, Mannheim, Germany), 1mM dNTP mix, 4µl of 5X RT-buffer, deionized distilled water, and 5µl RNA extract, which

were incubated at 55˚C for 30 minute, followed by heating at 85°C for 5 minute to inactivate the enzyme.

The amplification reaction was performed with a 50µl reaction volume consisting of 10X PCR Taq Buffer, 1.5 mM MgCl2, 200 µM dNTP mix, 25 pmol of each Beg9 and End9 primers, 2 U Taq DNA polymerase (Fermentas Life Sciences), and 5 µl cDNA. The thermal cycling conditions were as follows: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 1 minute denaturation at 94°C, 2 minutes annealing at 42°C, and 1 minute extension at 72°C. The final extension step was carried out at 72°C for 10 minutes. PCR products were analyzed on an agarose gel stained with ethidium bromide and visualized in ultraviolet transilluminator. The same RT-PCR protocol was used for P genotyping of rotavirus with consensus primers Con2 and Con3 amplifying VP4 gene (876bp).

### *Semi-nested multiplex PCR for rotavirus specific G and P genotyping*

Rotavirus specific G and P genotyping was performed using a semi-nested type specific multiplex RT-PCR test that detects seven G types and six P types (8-10). Briefly, in order to identify the specific G type, the amplicons obtained by consensus PCR, type specific primers (G1, G2, G3, G4, G8, G9, and G10) and consensus primer RVG9 for the VP7 gene were used in semi-nested type specific multiplex RT-PCR. For P genotyping, the amplicons obtained by consensus PCR, type specific primers (P4, P6, P8, P9, P10, and P11) and consensus primer Con3A for the VP4 gene were used (8-10). Both G and P genotyping protocols were identical and performed with a 50µl reaction volume consisting of 5 µl of 10X PCR Taq Buffer (100 mM Tris-HCl [pH 8.8], 500 mM KCl), 2 mM MgCl2, 200 µM dNTP mix, 20 pmol of each type specific primers and consensus primer, 2 U Taq DNA polymerase (Fermentas), and 2 µl product of first round PCR. The thermal cycling conditions were as follows: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of amplification (1 minute at 94°C, 2 minutes at 42°C, 1 minute at 72°C for each cycles), and a final extension of 10 minutes at 72°C. All amplified products of G and P genotypes were examined by gel electrophoresis in 2% agarose gel.

### *Statistical analyzes*

The dependent factor in our study was defined as "rotavirus positivity". The independent variables tested were sex, age, symptoms include diarrhea, duration of diarrhea, number of daily bowel movements, vomiting, daily number of vomiting, fever, dehydration, and hospitalization. Chi-square test ($\chi 2$) was used for statistical comparison using EpiInfo 6.0 software. The p-value less than 0.05 were accepted as significant with 95% of confidence**.**

## Results

From a total of 846 stool specimens analyzed, 144 (17%) samples were positive for rotavirus antigen. The majority of rotavirus positive cases (71.5%) were under 12 months of age (Table 1). Rotavirus positivity was more frequently observed in age group of 6-12 months among both males ($\chi 2$=15.52; p=0.00043) and females ($\chi 2$=10.86; p=0.0044), in cases with diarrhea lasting 3-4 days ($\chi 2$=12.12; p= 0.00234), with bowel movements of between 6-10 per day ($\chi 2$=17.75; p=0.00014), with vomiting ($\chi 2$=76.45, p<0.0001), with vomiting frequency of 5 or more daily ($\chi 2$=34.27; p<0.0001), in cases with dehydration ($\chi 2$=12.93, p=0.00032), and especially in those with severe dehydration ($\chi 2$=3.14, p<0.0001). In addition, hospitalization was more frequently observed among the children with rotavirus positivity ($\chi 2$=12.83, p<0.001), and rotavirus positive children were 1.96 times more hospitalized [Odds ratio (OR)=1.96 and 95% Confidence Interval (CI)=1.34-2.85]. Rotavirus positivity was not affected by sex ($\chi 2$=0.02; p=0.877), fever presence ($\chi 2$=0.46, p=0.499; Table 1).

According to the monthly distribution of rotavirus infection in children with acute gastroenteritis throughout the study period, higher positivity was observed in December (46 cases, 31.9%), November (25 cases, 17.3%), and January (23 cases, 16%), respectively (Figure 1).

The most prevalent rotavirus genotypes were G1P[8] (22.2%) and G1P[4] (17.3%), followed by G2P[4] (13.8%), G9P[4] (6.3%), G9P[8] (4.8%), G2P[8] (2.8%), G1P[10] (2.1%), and G4P[8] (1.4%) in 144 rotavirus positive cases (Table 2). Among rotavirus positive samples, the most common G genotype was G1 (41.7%), followed by G2 (16.6%), G9 (11.1%), and G4 (1.4%). The most frequently isolated P type was P[4] with the rate of 37.5%, followed by P[8] (31.2%) and P[10] (2.1%). Mixed rotavirus infections occurred in 29.2% (42) of cases (Table 3).

**Table 1.** Clinical and epidemiological features of children with and without rotavirus gastroenteritis.

| Data | Rotavirus positive Number (n=144) | %* (17.0) | Rotavirus negative Number (n=702) | %* (83.0) | *p*-value |
|---|---|---|---|---|---|
| **Sex** | | | | | *p*>0.05 |
| Male | 79 | 54.9 | 393 | 56.0 | |
| Female | 65 | 45.1 | 309 | 44.0 | |
| **Age groups** (month) | | | | | *p*<0.01** |
| 0-5 | 41 | 28.5 | 344 | 49.0 | in both sexes |
| *6-12* | *62* | *43.0* | *175* | *24.9* | (with no |
| 13-24 | 33 | 22.9 | 68 | 9.7 | difference |
| 25-36 | 3 | 2.1 | 30 | 4.3 | between sexes |
| 37-48 | 2 | 1.4 | 22 | 3.1 | *p*>0.05) |
| 49-60 | 3 | 2.1 | 63 | 9.0 | |
| **Symptoms** | | | | | |
| **Diarrhea** | 144 | 100 | 702 | 100 | --- |
| Duration of diarrhea (day) | | | | | |
| 1-2 | 32 | 22.0 | 196 | 28.0 | |
| *3-4*** | *58* | *40.0* | *182* | *26.0* | *p*<0.01** |
| 5-7 | 54 | 38.0 | 324 | 46.0 | |
| Number of daily bowel movements | | | | | |
| 3-5 | 75 | 52.1 | 489 | 69.7 | |
| *6-10*** | *65* | *45.1* | *206* | *29.3* | *p*<0.001** |
| 11-15 | 4 | 2.8 | 7 | 1.0 | |
| ***Vomiting*** | *106* | *73.6* | *238* | *33.9* | *p*<0.0001** |
| Daily number of vomiting | | | | | |
| 1-4 | 47 | 44.3 | 174 | 73.1 | |
| *5-8*** | *39* | *36.8* | *55* | *23.1* | *p*<0.0001** |
| *9-12*** | *20* | *18.9* | *9* | *3.8* | |
| Fever | 75 | 52.1 | 341 | 48.6 | *p*>0.05 |
| ***Dehydration*** | *102* | *70.8* | *380* | *54.1* | *p*<0.001** |
| Mild | 63 | 61.8 | 319 | 84.0 | |
| Moderate | 15 | 14.7 | 40 | 10.5 | |
| *Severe*** | *24* | *23.5* | *21* | *5.5* | *p*<0.0001** |
| ***Hospitalization**** | *79* | *54.8* | *269* | *38.3* | *p*<0.001** |

*% values are column percentages

**statistically significant independent variables (with the significantly different categories written in italic)

**Table 2.** Distribution of rotavirus G and P types.

| | Number of specimens (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Genotype** | **P[4]** | **P[8]** | **P[4]+P[8]** | **P[10]** | **P[4]+P[10]** | **P[8]+P[10]** | **P[4]+P[11]** | **Total** |
| **G1** | 25(17.3) | 32(22.2) | 23(16.0) | 3(2.1) | - | - | 2 (1.4) | 85 (59.0) |
| **G2** | 20(13.8) | 4 (2.8) | 2 (1.4) | - | - | - | - | 26 (18.0) |
| **G9** | 9 (6.3) | 7(4.8) | 2 (1.4) | - | 2 (1.4) | - | 1 (0.7) | 21 (14.6) |
| **G4** | - | 2 (1.4) | - | - | - | - | - | 2 (1.4) |
| **G1+G9** | 3 (2.1) | - | 2 (1.4) | 2(1.4) | 1 (0.7) | 1 (0.7) | - | 9 (6.3) |
| **G2+G10** | 1 (0.7) | - | - | - | - | - | - | 1 (0.7) |
| **Total** | 58 (40.2) | 45 (31.2) | 29 (20.2) | 5 (3.5) | 3(2.1) | 1 (0.7) | 3(2.1) | 144 (100.0) |

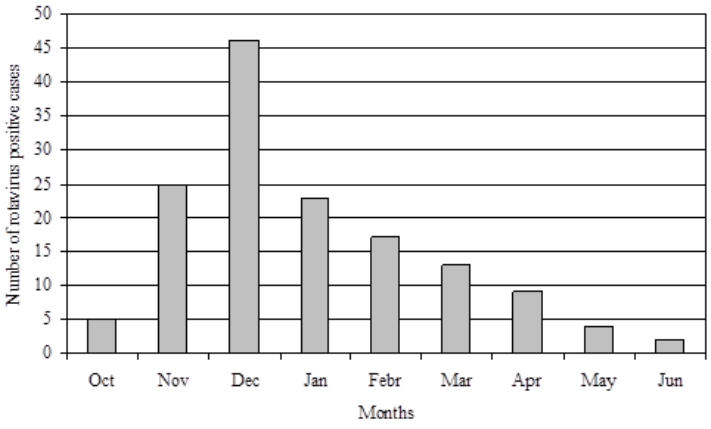


**Figure 1.** Monthly distribution of rotavirus positive and negative gastroenteritis cases.

**Table 3.** Distribution of rotavirus G-P genotype combinations of mixed infections.

| | Mixed infections | |
|---|---|---|
| **Genotype combinations** | **Number** | **%** |
| G1/P[4]P[8] | 23 | 16 |
| G1+G9 /P[4] | 3 | 2.1 |
| G2/P[4]P[8] | 2 | 1.4 |
| G9/P[4]P[8] | 2 | 1.4 |
| G1+G9/P[10] | 2 | 1.4 |
| G1+G9/P[4]P[8] | 2 | 1.4 |
| G1/P[4]P[11] | 2 | 1.4 |
| G9/P[4]P[10] | 2 | 1.4 |
| G9/P[4]P[11] | 1 | 0.7 |
| G2+G10 /P[4] | 1 | 0.7 |
| G1+G9/P[4]P[10] | 1 | 0.7 |
| G1+G9/P[8]P[10] | 1 | 0.7 |
| Total | 42 | 29.2 |

## Discussion

The high incidence of rotavirus gastroenteritis in both developed and developing countries suggests that the improvement of personal and public hygiene is not enough to prevent the spread of the disease. Therefore, vaccination is inevitable for prevention against rotavirus infections. Currently, there are two available rotavirus vaccines targeting protection against severe diarrhea, for reducing emergency admission rates, mortality, morbidity, and economic burden due to rotavirus infections (1, 6). Rotarix, monovalent vaccine against P1A[8]G1 genotype, is effective in protection against G1 type (96.4%), heterotypic G types G3 (93.7%), G4 (95.4%), and G9 (85%).13 However, the efficacy of Rotarix vaccine against heterotypic G2P[4] has been reported to be from 41% in Latin America (11), 77% in Brasil (12) to 85.5% in Europe (13).

The rate of rotavirus infection in children with acute gastroenteritis has been previously reported in varying proportions of 13-40.6% in Europe (14, 15), 17-44% in USA, (16, 17) 16-32% in Africa (18), 19.7% in Japan (19), 31.4-52% in China (20, 21), 6.3-35% in India (22) and 12.5-48.9% in Turkey (23-26). The frequency of rotavirus infection in our study was 17% (144 cases). The rates of rotavirus gastroenteritis are comparable in both developed and developing countries, but mortality from diarrhea is greater in developing countries as a result of inadequate treatment (1, 2).

In our study, the most common rotavirus G genotype was G1 with the rate of 41.7%, followed by G2 (16.6%), G9 (11.1%), and G4 (1.4%). The genotype G3, which is also one of the most common types worldwide, was not identified during the study period. Interestingly, the predominant P genotype was P[4] (37.5%), but not P[8] (31.2%). There is limited number of studies conducted on the distribution of rotavirus genotypes in Turkey. Reports in different years from Ankara (24) and Izmir (25) regions in Turkey also showed that G1 was the dominant genotype with 86% and 75.1%, respectively, but being higher than our finding that of 41.7%. We found G2 genotype as the second most common (16.6%) type, was higher than the rates of 3.3% and 0.8% reported in Ankara and Izmir, respectively (24, 25). Higher frequency of G1 genotype has also been consistent with previous results for rotavirus infection in France (61.7%) and Japan (72.7%) (27, 19). Our finding of 41.7% rate for G1 type was similar to that reported from Italy in 2008 (G1 40.7%), however the second most common type observed in Italy was G9, but not G2 (28).

The most prevalent genotype combination in our study was G1P[8] (22.2%) which was also identified as predominant genotype in the previous studies (14,28-30). The other genotypes were found to be G1P[4] (17.3%), G2P[4] (13.8%), G9P[4] (6.3%), G9P[8] (4.8%), G2P[8] (2.8%), G1P[10] (2.1%), and G4P[8] (1.4%), respectively. In contrast with these findings, Çataloluk et al. reported the genotype G4P[8] as the predominant type (42.2%) and G1P[8] as the second most common genotype (26.6%) circulating in Gaziantep region of Turkey in 2005 (31). However, another study indicated that G3P[8] (38.9%) was the most frequent genotype in Ankara from April 2009 to February 2010 (32). As distinct from the other studies (30, 33), the combinations of G genotypes with P[4] represented relatively high frequencies of G1P[4] (17.3%), G2P[4] (13.8%), and G9P[4] (6.3%) in our study. The rates for G1P[4], G2P[4], and G9P[4] genotypes in Europe were 0.29%, 10% and 0.19% respectively (30). The frequency of G9P[8] was 4.8% in our region. Whereas, G9P[8] was detected to be the most common type of rotavirus-associated gastroenteritis in Poland (71.1%) and Spain (87.7%) (14). G9P[8] was reported as the second most common genotype (10.1%) in Istanbul, Turkey (29). Our finding of G2P[8] was 2.8%, while Ittiruza et al. found G2P[8] as 0.47% in Europe (30). The presence of genotype G1P[10] (2.1%), which might be due to possible reassortment between human and animal rotavirus strains, was reported in our study for the first time in Turkey. This genotype was observed in Europe with 1.7%.30 The globally common G1P[8], G2P[4], G4P[8], and G9P[8] genotypes constituted 42.7% of rotavirus strains, but G3P[8], which is also another common genotype, was not detected in our study. The percentage of mixed rotavirus infection in the present study was quite high (29.2%) compared to those reported in previous studies (28-31). These findings confirm that rotavirus genotype distribution varies between regions, countries, and also differs from year to year.

**Conclusion**

In conclusion, although the predominant rotavirus genotypes circulating during the study period in our region are the targets of current rotavirus vaccines, uncommon, non-vaccine rotavirus genotype combinations such as G1P[4] (17.3%) and G9[4] (6.3%) were also detected. The high rate of mixed rotavirus infections (29.2%) in the study population might increase emergence of new strains due to natural reassortment, which might decrease the effectiveness of current rotavirus vaccines against rotavirus infections. Therefore, continuous prospective monitoring of circulating strains of rotavirus is essential to evaluate the efficacy and convenience of rotavirus vaccines.

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# Prevalence of *cagA* and *babA2* positive *Helicobacter pylori* strains in dyspeptic patients in Iran

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## Abstract
**Background:** *Helicobacter pylori* genome encodes a large number of virulence and adhesion factors that involved in bacterial adhesion to host cells and exerts its virulence effect with CagA secretion. In this study, we aimed to examine the relationships between *babA2* and *cagA* genotype and presence of gastric disorders in patients from Iran.
**Material and Methods:** The presence of *H. pylori* and selected genes (*cagA* and *babA2*) were detected by PCR method from the genomic DNA of 105 patients who had been diagnosed with gastric disorders like chronic gastritis by endoscopic and histopathologic routes.
**Results:** Presence of *H. pylori* (*glmM gene*) was detected in 85 out of 105 (80.9%) patients. According to our results *cagA* gene was found in 66 out of 85 (77.6%) patients and *babA2* gene was found in 80 out of 85 (94.1%) patient.
**Conclusion:** In conclusion, the *cagA* and *babA2* genotypes might be considered as useful biomarkers for non-ulcer disease (NUD) patients and gastric disorders in the geographic region of Iran and the presence of *H. pylori* strains with double-positive status is high clinical relevance to *H. pylori*-associated diseases.

**Key words:** *Helicobacter pylori*, babA2, cagA, gastric disorders.

## Introduction
Less than three decades ago, Robin Warren and Barry Marshall definitively identified *Helicobacter pylori* by culturing an organism from gastric biopsy specimens that had been visualized for almost a century by pathologists (1). *H. pylori* is a gram-negative spiral organism that is capable of colonizing the gastric mucosa and form the main cause of chronic active gastritis (2). Colonization with *H. pylori* is the commonest infection worldwide, affecting at least half the world's population (3). Adherence of *H. pylori* to the gastric epithelium facilitates initial colonization, persistence of infection, and delivery of virulence factors to host epithelial cells.

The cytotoxin-associated gene (*cagA*) was the first gene found to be differentially present in *H. pylori* isolates and is considered a marker for the presence of the cag pathogenicity island *cagPAI*. *H. pylori* strains can be divided into CagA positive or negative strains. CagA was identified as the first protein of the *cagPAI* and appeared to be a major virulence factor (4). CagA is an oncoprotein that thought to be involved in cancer development. The recently described blood group antigen-binding adhesin BabA has been shown to mediate adherence of *H. pylori* to Lewis b receptors on gastric epithelium (4).

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Albeit three bab alleles have been recognized (babA1, babA2, and babB), only the babA2 gene product is intransitive blood-group antigen-binding adhesin that mediates attachment of *H. pylori* to human Lewis-b antigens (5, 6). In this investigation, we focused on *H. pylori* BabA2 adhesion factor and CagA virulence factor in patients suffering from chronic gastritis. We sought to examine the relationships between *babA2* and *cagA* genotypes in patients with gastrointestinal disorders and identify the independent markers of non-ulcer disease (NUD) such as dyspepsia and chronic gastritis in patients from Iran.

## Material and methods

### *Sample collection*

In this study, a total of 105 biopsy samples from the stomach antrum and corpus were obtained from patients with gastro duodenal disorders like chronic gastritis, dyspepsia and non-ulcer disease (NUD) that, referred to endoscopy department of Imam Reza hospital, Tabriz, Iran. For study the babA2 and cagA genes, PCR method was used. To PCR analysis DNA was extracted from obtained biopsy samples by performing QIAamp DNA Mini Kit (Qiagen Inc., Germany), according to the manufacturer‘s instructions and prepared DNA were stored at −20 °C for using in future studies.

### *PCR analysis*

The *glmM* gene was primarily amplified for detection of *H. pylori* DNA in collected samples. The forward primer (5′-AAG CTT TTA GGG GTG TTA GGG GTT T-3′) and the reverse primer (5′-AAG CTT ACT TTC TAA CAC TAA CGC-3′ ) were derived from the published "*H. pylori* " glmM (Urea C) sequence and used to amplify a 294-bp segment of *H. pylori urease C gene* (7). PCR analyses were carried out to determine the presence or absence of *cagA* and *babA2* genes in each *H. pylori* positive sample by using specific primers. The sequences of these primers are described in (Table 1).

The amplification reaction consisted of 1 to 2 µl DNA samples in a final volume of 50 µl containing 1× PCR buffer, 200 µM (each) deoxy nucleoside triphosphate, 100 pmol of primers and 2.5 U of Taq DNA polymerase in Thermo cycler (MJ Mini BIO-RAD).

For analysis of the amplified products of each PCR assay, 6 µl of the amplicons were electrophoresed with a 1X tris-acetate-EDTA buffer on 2% agarose gel stained by ethidium bromide (5 µl/100ml). The amplicons were visualized by UV transillumination, and a 100 base pair ladder was used as standard.

The statistical analysis of data was accomplished by using logistic regression, chi-square test with significance set at a P value of $< 0.05$.

## Results

From all of 105 patients, *H. pylori* (presence of *glmM* gene) were detected in 85 patients (80.9%) including 44 (41.90%) men and 61 (58.09%) women with mean age of 53 years old (±30.5). Age, sex, and PCR findings of patients were tabulated and analyzed by Matlab and SPSS 14 statistical package. In our study *cagA* and *babA2* genes were found in 77.6% and 94.1% of *H. pylori* positive samples respectively. The prevalence of *cagA* and *babA2* harboring strains in Iran is similar. The results of this study suggest that, in Iran like some other countries the presence of *cagA* and *babA2* positive *H. pylori* strains is associated with *H. pylori* related diseases such as dyspepsia, NUD and chronic gastritis (Table2).

**Table 1.** Description of the pairs of primers used in the amplification of *cagA* and *babA2* genes.

| Gene/ Primers | Sequence | Size/ (References) |
|---|---|---|
| ***glmM*** | **F** 5'-AGCTTTTAGGGGTGT TAGGGGTTT-3' **R** 5'-AAGCTTACTTTCT AACACTAACGC-3' | 294 bp (7) |
| ***cagA*** | **F 5'**-GATAACAGGCAA GCTTTTGAGG-3' **R 5'**-CTGCAAAAGATT GTTTGGCAGA-3' | 349 bp (7,16) |
| ***babA2*** | **F 5'**-AATCCAAAAAG GAGAAAAAGTATGAAA-3' **R 5'**-TGTTAGTGATTTCG GTGTAGGAC-3' | 832 bp (15) |

**Table 2.** The statistical analysis and frequency of *babA2* and *cagA* positive *H. pylori* strains.

| Genotype | NUD n (%) | $\chi^2$ | *p* value |
|---|---|---|---|
| ***babA2* positive** | 80/85 (94.11) | 9.509 | 0.0020 |
| ***cagA* positive** | 66/85 (77.64) | | |

## Discussion

In the study of infectious diseases, researchers for recognize a real pathogen from innocuous organisms, often focus on bacterial virulence factors. Since the discovery of *Helicobacter pylori*, many investigations have focused on explaining the microorganism pathogenicity mechanisms that are associated with disease outcome. In studies conducted on *H. pylori*, several virulence factors have been discussed. But one of the most critical property of *H. pylori*, is its ability to remain for many years within the host tissues without any damage. Therefore, colonization is not equivalent with virulence but rather may refer to the persistence of the microorganism in a specific site in the host. The adherence of *H. pylori* to host cells is a pertinent step in the development of gastro duodenal disorders. In our study, we focused on *H. pylori* BabA2 adhesion factor and CagA virulence factor in patients suffer from gastro duodenal disorders like chronic gastritis and dyspepsia. In our study *cagA* and *babA2* genes were found in 77.6% and 94.1% of *H. pylori* positive samples respectively. The prevalence of *cagA* and *babA2* harboring strains in Iran is similar.

In studies of *H. pylori* colonization and its adhesion to the host cells, the BabA adhesion has been the most studied factor (8, 9). BabA2 attaches *H. pylori* to host epithelial cells, permitting the transfer of CagA toxin to the gastric epithelium and so increasing gastric tissue damage. Some authors suggest that, the presence of *babA2* is associated with some disorders like duodenal ulcer and gastric cancer, when found in conjunction with *vacA s1* alleles and cagA (10). In our study *cagA* gene was found in 77.6% of patients with *H. pylori* and *babA2* genotype was positive in 94.1% of patients, we also showed that, the co-presence of these genes can increase the risk of gastro duodenal disorders. The last analyses of *babA2* gene as a marker for diagnosis *H. pylori* associated disease have produced inconsistent data on the useless of this gene expression in anticipating clinical outcomes, which is most likely related with the geographic scope of the *H. pylori* strains. Another author suggests that In Thai populations, *babA2* is not a biomarker for peptic ulcer disease or chronic gastritis (11). But, for strains isolated from Turkey, northern Portugal or Germany, BabA2 expression is related to the severity of gastric duodenal disorders (12, 13 and 14). *H. pylori* isolates from Cuba displayed a high frequency (82.3%) of the *babA2* allele in Cuban patients (15). Our data suggests that in Iran like some other countries the presence of *cagA* and *babA2* positive *H. pylori* strains is associated with *H. pylori*-related diseases such as chronic gastritis and there is a significant association between increased two genes together.

## Conclusion

In conclusion, the *cagA* and *babA2* genotypes might be considered as useful markers for monitoring and prognosis of NUD patients and gastric disorders in the geographic region of Iran and the presence of *H. pylori* strains with double-positive status is of high clinical relevance to *H. pylori* associated diseases.

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# Investigation of liver autoantibodies in anticentromere antibody positive patients: A preliminary research

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## Abstract

**Background:** Anticentromere antibody (ACA) is regarded to be a serological marker specific to CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactylia, and telangiectasia) syndrome. ACA is also found in the sera of patients with autoimmune liver disease. In the present work, anti-soluble liver antigen\liver pancreas antigen (anti-SLA\LP), anti-liver cytosolic antigen 1 (anti-LC1), anti-liver kidney microsomal antigen 1 (anti-LKM1), and anti-mitochondrial antibody M2 (AMA-M2) were evaluated in the patients who had positive anticentromere antibody.

**Material and Methods:** A total of 39 patients who were positive anticentromere antibody were enrolled in this study undertaken in the Izmir Katip Celebi University, Ataturk Training and Research Hospital, Microbiology laboratory between January and September 2015. Positive anticentromere antibody and liver autoantibodies were analyzed. Anticentromere antibody and liver autoantibodies were studied by indirect immunofluorescence method (IIF) and immunoblotting method (IB), respectively. The patients who had negative anticentromere antibody were used as a control group.

**Results:** According to the study's results, positivity was detected in 3 of 39 patients (%7.6) in terms of liver autoantibodies, all of which were AMA-M2. There was no statistically significant difference between ACA and autoimmune liver autoantibodies.

**Conclusion:** In this study, we reported our preliminary experience to provide evidence for the detection of various autoantibodies as potential diagnostic or prognostic tests. Further studies that contain a broad range of patients may contribute to the field.

**Key words:** Anticentromere antibody, autoimmune hepatitis, immunofluorescence method.

## Introduction

Anticentromere antibody (ACA) is regarded to be a serological marker specific to CREST (calcinosis, Raynaud's phenomenon, esophageal dysmotility, sclerodactylia, and telangiectasia) syndrome (1-4). In addition to being present in CREST syndrome, ACA is also detected in the sera of patients with autoimmune liver disease (5).

In the present study, anti-soluble liver antigen\liver pancreas antigen (anti-SLA\LP), anti-liver cytosolic antigen 1 (anti-LC1), anti-liver kidney microsomal antigen 1 (anti-LKM1), and anti-mitochondrial antibody M2 (AMA-M2) were evaluated in the patients who had positive anticentromere antibody.

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## Material and methods

A total of 39 patients who had positive anticentromere antibody were enrolled in this study undertaken in the Izmir Katip Celebi University, Ataturk Training and Research Hospital, Microbiology laboratory between January and September 2015. The patients who had negative anticentromere antibody were used as a control group. Positive anticentromere antibody and liver autoantibodies were analyzed retrospectively. Anticentromere antibody and liver autoantibodies were studied by indirect immunofluorescence method (IIF) and immunoblotting method (IB), respectively. Blood samples were collected in serum separator tubes, centrifuged at 2,500 rpm for 10 min. ANA patterns were evaluated by using the HEp-2010/Liver (Monkey) indirect immunofluorescence assay kit (Euroimmun AG, Lübeck, Germany, Lot No. F160318DI). Serum samples were processed in a dilution of 1:100 and conjugated with specific anti-human IgG (Euroimmune AG, Lübeck, Germany, Lot No. F160318DI). The fluorescence intensity was scored at x 400, semi-quantitatively from 1+ to 4+ relative to the intensity of the positive (4+) and negative control. The test result was discarded if the positive control sample failed to show the precise results. There has not been any duplication in assessing the samples. In immunoblotting method liver profile (Euroimmune AG, Lübeck, Germany, Lot No. D150327AD) that contains nylon strips coated with AMA M2, LKM-1, LC-1, SLA/LP antigens along with a control band was used (Figure1).

***Statistical analysis***

The difference between variables was analyzed by the statistical Chi square test. Statistical analyses were performed using SPSS version 22.0 for Windows (SPSS Inc., Chicago, IL, USA). A p-value <0.05 was considered to be significantly different.

## Results

The patients (1 male and 38 females) were aged between 28-80 years, an average of 56.7±11,4 years. Among ACA-positive patients (Figure 2), 16 (41%) systemic sclerosis (SSc), 12 (30.8%) mixed connective tissue disease (MCTD) and 11 (28.2%) CREST diagnoses were detected. AMA-M2 positivity was detected in 3 (7.6%) of 39 ACA-positive patients (Table 1). All of the positive liver autoantibodies were AMA-M2 antibodies and all of the positive patients were women. There was no statistically significant difference between ACA and autoimmune liver autoantibodies (p>0.05).

**Table 1.** Diagnosis and the number of positive liver autoantibody results of the patients.

| | **AMA-M2** | **LKM-1** | **SLA/LP** | **LC-1** |
|---|---|---|---|---|
| **CREST syndrome** | 2 | 0 | 0 | 0 |
| **Systemic sclerosis** | 0 | 0 | 0 | 0 |
| **MCTD** | 1 | 0 | 0 | 0 |
| **Total** | **3** | **0** | **0** | **0** |

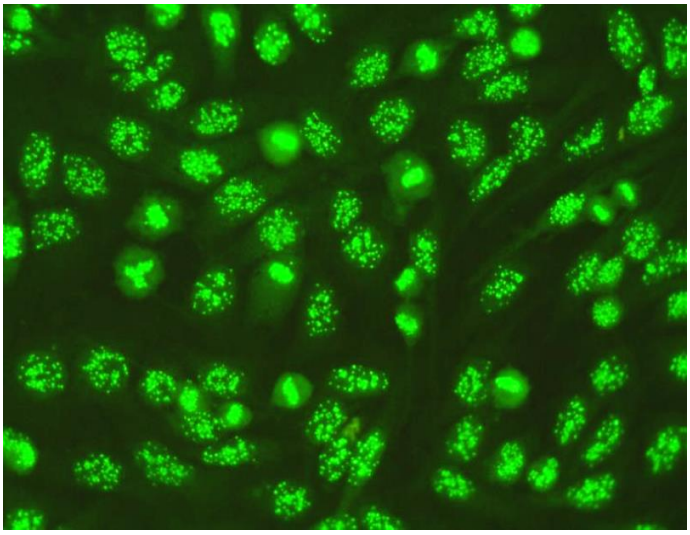

**Figure 2.** Anticentromere pattern (4+) in IIF.

## Discussion

Rheumatoid diseases are multisystem inflammatory diseases related with the development of autoantibodies to several self-antigens. The liver may be one of the organs affected by these diseases (6). ANA molecular targets specific for autoimmune hepatitis (AIH) are not present. A different profile of ANA positivity reminiscent of that found in Systemic Lupus Erythematosus (SLE) has been informed in AIH. However, at least a third of AIH patients positive for ANA do not react with known nuclear targets (7, 8). Himoto et al. have investigated clinical characteristics of patients with autoimmune hepatitis seropositive for anticentromere antibody. In the

study, the scoring system proposed by the International Autoimmune Hepatitis Group was used for diagnosis of AIH. Seropositivity for ACA was evaluated by a discrete speckled pattern on HEp-2 cells by an immunofluorescent method. Seropositivity for ACA was evaluated by a discrete speckled pattern on HEp-2 cells by an immunofluorescent technique. It was informed that eight (17%) of 47 patients with AIH had ACA. Statistically significant differences in age, sex, onset pattern of the disease, progression to hepatic failure and relapse rate were not present between the ACA-AIH and other-AIH groups. They reported that the frequency of concurrent autoimmune diseases in ACA-AIH was remarkably higher than that in other-AIH groups (9).

Lodh et al. presented case report associated with a 49-year-old type 2 diabetes mellitus patient with abdominal pain and black stool for 15 days. They informed that laboratory investigations provided diagnosis of AIH with anticentromere antibodies. The authors reported that screening AIH patients for anticentromere antibody is not obligatory, however, can be regarded, particularly in the presence of disease-related symptomatology for quicker, more reliable diagnosis and optimum management (10).

The Committee for Autoimmune Serology of the International Autoimmune Hepatitis Group (IAIHG) supplied guidelines on testing for autoantibodies associated with AIH. According to the guidelines IIF screening on fresh sections of liver, kidney, and stomach from rodents should be the first line screening and the use of the three tissues enabling concurrent detection of almost all the autoantibodies related to liver disease, namely, against smooth muscle antigen (SMA), ANA, LKM1, AMA, and LC1 (11).

You et al. informed that there have been only two reports in the literature considering investigations associated with anti-centromere antibodies in patients with AIH. The studies have indicated the presence of these antibodies at a low frequency of 3% in cases of AIH. The authors reported that the frequency of the discrete speckled pattern of ANA (related to ACA) is increased in AIH patients with ACA, supporting the close association between the AIH and SSc in the Japanese population. They emphasized that no certain consequences can be drawn from these findings, and additional and advanced studies are necessary to examine the relationship between ACA and AIH (12). There are a lot of investigations associated with the clinical significance of ACA in literature (13-16).

## Conclusion

Although, our current study is a preliminary research, we reported our experience in order to provide evidence for the detection of various autoantibodies as potential diagnostic or prognostic tests. Well-designed further studies that contain a broad range of patients may contribute to the field.

**Ethics Committee Approval:** Ethics Committee approval was received for this study from the ethics committee.

**Informed Consent:** Written informed consent was obtained from patients who participated in this study.

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*J Immunol Clin Microbiol*

# Early Therapeutic Penetrating Keratoplasty for Fungal Keratitis

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***Abstract***
**Background:** To present two cases who developed fungal keratitis after an organic trauma and who were treated with therapeutic penetrating keratoplasty in the early period.
**Case presentations:** A 14 years old female and a 63 years old male patients came with the complaints of pain after organic trauma. Fungal keratitis was diagnosed in both two cases. TPKP was performed on the 5th day of the topical antifungal treatment (Case 1), on the 5th day of the topical antifungal treatment (Case 2) and 7th day of the trauma (both two cases). Graft transparency was protected in the post-operative period; no recurrence or fungal reactivation was detected in two patients.
**Conclusion:** The study has proven that TPKP performed in the early period of fungal keratitis enables rapid infection eradication and rapid visual success.
**Key words:** Fungal keratitis; Early Therapeutic Penetrating Keratoplasty; Organic trauma

## Introduction

The most common types of fungal keratitis, which is one of the most frightening diseases in ophthalmology, are filamentous fungi (Fusarium types, Aspergillus types) and yeast fungi (Candia types, Cryptococcus types). Filamentous species were the most common causative pathogens (1). Most studies report that traumas caused by plant and organic material are the most common causes of fungal keratitis. Other risks include use of contact lens, use of topical steroids and antibiotics, corneal surface diseases (herpes keratitis, keratitis sicca, atopic keratoconjunctivitis, recurrent erosion), eye surgery (reactive surgery, keratoplasty, loose corneal sutures), immune suppression, and diabetes (2). Early stages of the infection might be controlled by appropriate antifungal agents, but therapeutic penetrating keratoplasty (TPKP) is the treatment of choice for intractable cases (3).

In most cases after TPKP the pathogen is successfully eradicated by surgery and the development of endophthalmitis, orbital cellulitis, or even panophthalmitis is avoided.

This study aims to present two cases who developed fungal keratitis after an organic trauma and who were treated with TPKP in the early period.

## Case 1

The fourteen-years old female patient came with the complaints of pain, redness, and reduced vision after something got into her left eye while working in the field two days before. Visual acuity (VA) was 0.0001 in the left eye. Intraocular pressure (IOP) was normal digitally. In the central left eye cornea, there was suppurative lesion, corneal thinning, and hypopyon (Figure 1a). Posterior segment was normal by ultrasound examination.

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Corneal scrapings obtained were inoculated onto 5% blood agar, EMB (Eosin Methylene Blue) agar, chocolate agar, and SDA (Sabouraud dextrose agar) media. Plenty of hyphal was observed in the direct microscopic examination. Fungal keratitis was diagnosed. The treatment was started with Fortified vancomycin (50 mg/ml), Fortified ceftazidime (50 mg/ml), and amphotericin B 0,15 % in hourly doses.

Natamycin pomade was not given because it is not available in our country. No reduction in the size of the lesion was detected on the 4th day of the treatment (Figure 1b). On the contrary, due to the extension of infiltrates through limbus, topical voriconazole was aimed, but it could not be obtained. Then, TPKP was performed with the patient under general anesthesia on the 7th day of the trauma and on the 5$^{th}$ day of the topical amphotericin B 0,15 % treatment.

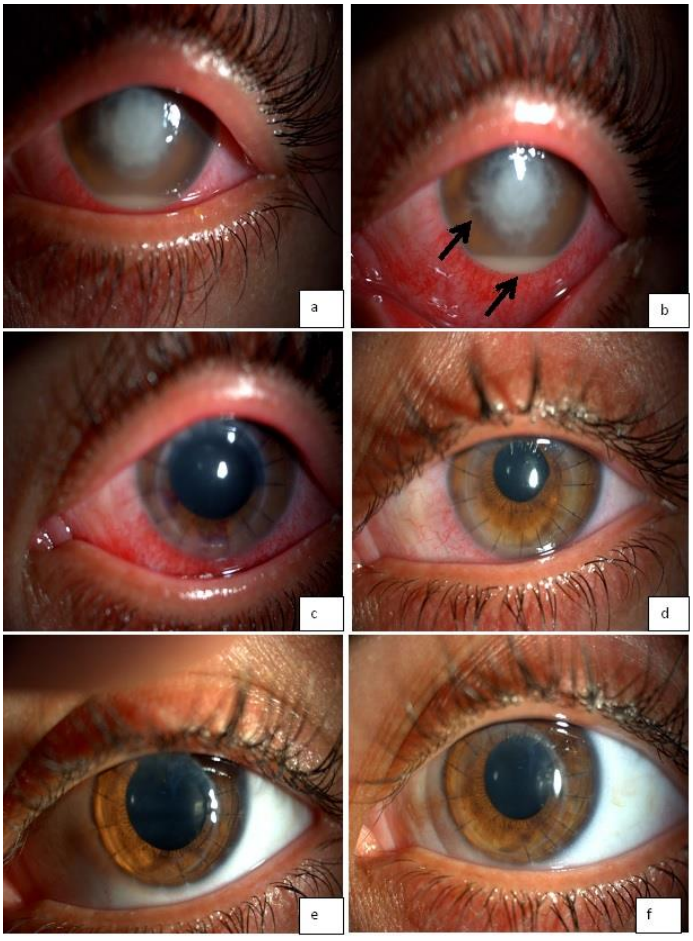


**Figure 1.** Figures of case 1 (a) arrival at the hospital, before topical amphotericin B treatment, (b) Five days after topical amphotericin B, before surgery, an increase of hypopyon and infiltrate, (c) first day after TPKP, (d) one month after TPKP, (e) three months after TPKP, (f) nine months after TPKP (TPKP: Therapeutic penetrating keratoplasty).

Excision of the recipient cornea was performed with 7.75 mm vacuum trepan in the size involving all infective corneas. The membranes covering the iris surface and angle in anterior chamber were removed. The tissue, which was excised was sent for microbiology evaluation. Anterior chamber and margin of the recipient cornea were washed with amphotericin B (0.05/1 cc). Then, a new operation table and set were prepared; 8.25 mm size donor cornea was sutured to the recipient bed, one by one and using 10/0 nylon suture. 0,005mg/0,1cc amphotericin B was performed by intracameral.

Following the operation, acetazolamide 250 mg tablet (twice a day), topical moxifloxacin (six times a day), topical amphotericin B 0,15 % (eight times a day), artificial tears (eight times a day), topical cyclosporine 0,05% drop (four times a day) methylprednisolone tablet 32 mg/day, lansoprazole tablet (once a day) was started.

There was culture growth in the post-operative period. Type description of the strain was performed using VITEK® MS MALDI-TOF (Matrix assisted laser desorption ionization time of flight mass spectrometry) version 2.0 system. Type description was reported 99.4% as *Fusarium solani* (Figure 2 a-d).

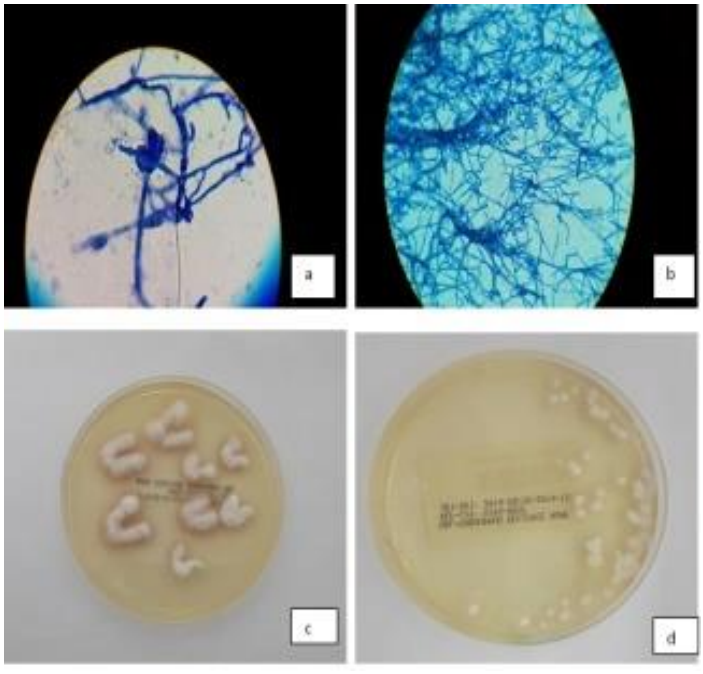


**Figure 2.** Staining by methylene blue of *Fusarium solani* (a, b) and identification by Matrix assisted laser desorption ionization time of flight mass spectrometry, culture media (c, d).

Moxifloxacin, methylprednisolone (in tapering doses) and lansoprazole tablet was stopped in the third post-

operative week. Dexamethasone drops (two times a day) was included in the treatment and continued in increasing doses. Topical amphotericin B 0,15 % was continued in tapering doses until the third post-operative month.

Graft transparency was found to continue until the 9th month of the follow-ups, no recurrence or fungal reactivation was detected (Figure 1c-f). Best corrected visual acuity (BCVA) was 0.8 in the postoperative 9th month; and IOP was normal.

## Case 2

63 years-old male patient came to the clinic with the complaint that a walnut fell on his left eye a day before. VA was 0.03 in the left eye. IOP was normal digitally. In the central left eye cornea, there was infiltrative lesion, corneal thinning, and hypopyon (Figure 3a). Posterior segment was normal on ultrasound examination. Fungal was positive in direct microscopic examination, but no growth was displayed in culture.

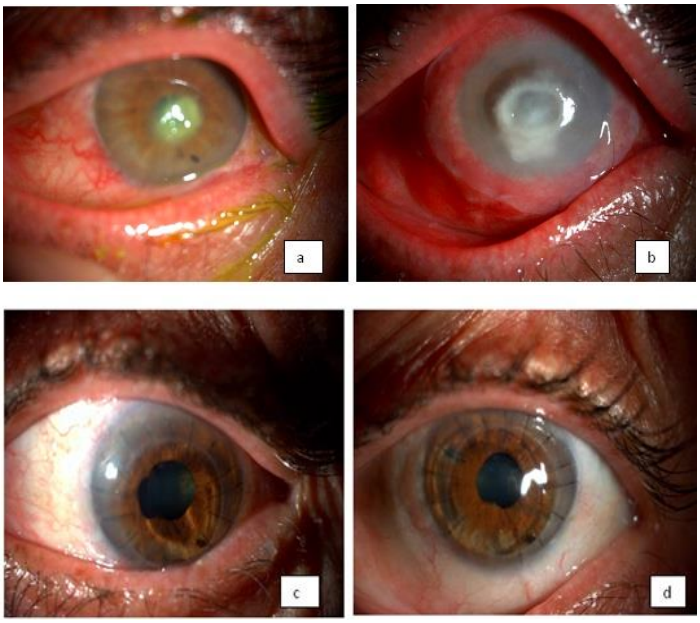


**Figure 3.** Figures of case 2 (a) First day after trauma and before treatment, (b) 4 days after topical, intracameral voriconazole treatment, and AMT, before TPKP, (c) 1 month after TPKP, (d) 11 months after TPKP (AMT: Amniotic membrane transplantation, TPKP: Therapeutic penetrating keratoplasty)

The treatment was started with hourly doses of Fortified vancomycin (50 mg/ml), Fortified ceftazidime (50 mg/ml), amphotericin B 0,15 %. However, because there was no response to the present treatment and progress, the antifungal was changed on the second day. Topical voriconazole (1%) was given hourly. Besides, intrastromal and intracameral (0.1mg/0.1cc doses) voriconazole, amniotic membrane transplantation (AMT) was applied due to central corneal thinning. There was no response to the treatment (Figure 3b). TPKP was performed under local anesthesia on the 4th day of the topical voriconazole treatment and 7th day of the trauma.

Differently from Case 1, preoperative anterior chamber and recipient corneal margin was washed with voriconazole (0.1 mg/0.1cc) and 0,1 mg/0,1cc voriconazole intracameral was performed. Medication in the post-operative period was done similarly to the medication in Case 1. Graft transparency was protected in the post-operative period; no recurrence or fungal reactivation was detected (Figure 3c, d). BCVA was 0.5 with a grade 2 nuclear sclerosis in the postoperative 11th months; and IOP was normal.

## Discussion

Corneal fungal infections form between 6 to 53% of ulcerative keratitis. Fungal keratitis are generally related with traumas caused by plants or metals contaminated with fungi; and they are generally formed through the medium of filamentous fungi. Trauma can be too weak for the patient to remember (2). In both cases the present study had an organic trauma history.

In India, 10.7% out of 264 fungus keratitis were found to have Fusarium solani (4). The Fusarium proportion was found between 45 and 67 % in studies conducted in Brazil (5). The present study has also detected Fusarium in Case 1, but no culture growth was displayed in the other case.

If there is hyphae and filamentous fungus growth in culture, topical 5 % natamycin seems to be the optimal medicine, and topical 0.15% amphotericin-B is an alternative. As natamycin is not available in our country, amphotericin-B was used as the first option in this study.

Surgery is needed in cases which show progress despite maximum antifungal treatment, which have an extension tendency to limbus, and which have perforation threat. In a study conducted in Brazil, 22 cases out of 41 (54%) needed PKP; and 19 of these 22 cases were observed to have a perforation and 3 of them had no response to the medical treatment (5). Oechsler et al. Investigated 52

patients with fungal keratitis, performed PKP in 7 patients, and detected fusarium in all these 7 patients. Of these 7 patients, 4 of them were found to have a perforation and 3 of them gave no response to the medical treatment (6). In the present study early surgical intervention was preferred for both cases because of severe pain, no response to the treatment, and the tendency of infiltrates to extend to the limbus.

Use of corticosteroid in the post-operative period requires a great deal of attention in order to prevent graft reaction. Inflammation of corticosteroid infection or the probability of causing super infection should be kept in mind. Perry et al. reported that use of cyclosporine-A after PKP (0.5%) is a good alternative to corticosteroid (7). In this study, topical steroid was not used in the first three post-operative weeks. Later on, it was applied in increasing doses and under the umbrella of a topical antifungal. Topical cyclosporine-A (0.05%) was used as of the first post-operative day.

Recent studies have reported that early surgery performed without waiting perforation and the extension of the lesion to limbus has better outcomes in resistant cases (8,9). Rapid visual success, symptomatic relief, and shorter hospital stay have been achieved by the early surgery intervention in the cases in this study. Both cases were discharged from the hospital on the third post-operative day.

## Conclusion

Consequently, with the two cases diagnosed with fungal keratitis, the present study has proven that TPKP performed in the early period enables rapid infection eradication and rapid visual success.

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*J Immunol Clin Microbiol*

# Autoimmune extraintestinal manifestations of *Helicobacter pylori* infection: A bundle of conflicts

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## Abstract

It has been well-known that several microorganisms that have an effect on particular areas of the body might additionally have systemic sequelae. In the last ten years, various studies have been performed on the relationship between *Helicobacter pylori* infection and a variety of extra digestive illnesses including immunological, hematological, neurological disorders and different pathologies. It has been recommended that complicated interactions between bacterial and host genetic factors, as well as environmental factors, play considerable roles in determining different clinical outcomes. Although, there are conflicting and controversy data in some diseases, in the light of literature, it is currently accepted; that the presence or absence of *H. pylori* infection might influence the chance of developing of many autoimmune diseases. Treatment of *H. pylori* infection has been reported to be effective in some diseases like Schoenlein-Henoch purpura, ITP, psoriasis and chronic autoimmune urticaria. This review focuses the possible role of *H. pylori* infections in various autoimmune diseases taking into account the recent literature.

**Key words:** *Helicobacter pylori*, extraintestinal manifestations, autoimmune diseases

## Introduction

***H****elicobacter pylori* chronically infects more than half of the world's population and, one of the most frequent causes of gastrointestinal infections and it is estimated that the pathogen has co-evolved with its human host for at least 30.000 years (1-5). *H. pylori* infection fulfills each of Koch's postulates as an infectious agent inflicting chronic active gastritis and ulcer. It is related with a wide spectrum of gastrointestinal diseases, as well as gastroduodenal ulcers, mucosaassociated lymphatic tissue lymphoma (MALToma), and gastric adenocarcinoma.

It is well-known that several microorganisms have an effect on particular region of the body might additionally have systemic sequelae like Campylobacter species *Streptococcus pneumonia* infections.

In the last decade, various studies have been performed on the relationship between *H. pylori* infection and a variety of extra digestive illnesses, including immunological, hematological, neurological disorders and different pathologies (Table 1). More recently, numerous publications have supported a role for *H. pylori* infection in causing a variety of extraintestinal manifestations like allergic, chronic inflammatory and autoimmune diseases. It has been recommended that complicated interactions between bacterial and host genetic factors, as well as environmental factors, play considerable roles in determining different clinical outcomes among different subjects (1).

This review focuses the possible role of *H. pylori* infections in different autoimmune diseases by a systemic approach.

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### *Helicobacter pylori,* infections and possible pathophysiological mechanisms

The agent is a Gram-negative spiral shaped bacterium that has the unique ability to colonize the human gastric mucosa in spite of acid pH of the stomach. Virulence factors like urease enzyme and flagella are present in all infectious strains and are required for colonization of the gastric mucosa. The other major virulence factors associated with pathogenicity are: cytotoxin-associated gene A (CagA) and Vacuolating cytotoxin A (VacA) toxins (1). The *cagA* and *s1/m1 vacA* alleles have associated with a higher degree of gastric mucosal inflammation (6).

*H. pylori* infection is one of the most common bacterial infections worldwide and its prevalence has been estimated to extend from 40 to 80% and it changes widely by geographic region, age, race, ethnicity, and socioeconomic factors (1-3). Gastroduodenal ulceration and carcinogenesis are exclusive results of this infection.

Diversity in the clinic result of *H. pylori*-induced pathologies are multifactorial, involving a complex interplay between host immune responses and the pathogen virulence factors (1).

Several mechanisms have been suggested in an effort to clarify the extraintestinal manifestations of *H. pylori* infections. One of them is; gastric vascular permeability increases during atrophic gastritis due to infection and might cause increased exposure to alimentary antigens.

In addition, the gastric infection causes releasing of inflammatory mediators and molecular mimicry to systemic circulation. For example, antigastric autoantibodies have been found in more frequently in patients infected with *H. pylori* (7).

After the gastric colonization by *H. pylori*, production of large amounts of various proinflammatory substances, like interleukins, eicosanoids, and several proteins of the acute phase occur (8). This inflammatory response may cause the development of Ag-Ab complexes or cross-reactive antibodies due to molecular mimicry and may result in damage to other organs.

Also, it has been suggested that *H. pylori* induces a remarkable development similar to that seen in the molecular mimicry between *Streptococcus pyogenes* antigens and host proteins, resulting in both humoral and cell mediated immunologic reactions and eventually causing rheumatic fever, arthritis and rheumatic heart disease (7).

**Table 1.** Extraintestinal manifestations with a proven or suspected pathophysiological role in *H. pylori* infection.

| Affected system | Clinical manifestations |
|---|---|
| **Cardiovascular system** | Stroke, atherosclerotic heart disease, hypertension, Primary Raynaud phenomena |
| **Central Nervous system** | Alzheimer's disease, Parkinson's disease, migraine |
| **Immune system** | Rheumatoid arthritis, Immune thrombocytopenic purpura, Raynaud's phenomenon, Sjogren's syndrome, diabetes mellitus |
| **Endocrine system** | Autoimmune thyropathies, obesity |
| **Respiratory system** | Bronchial asthma, Lung cancer |
| **Hematologic system** | Iron deficiency anemia, Cobalamin deficiency |
| **Hepato-biliary diseases** | Hepatocellular carcinoma, Cholangiocellular carcinoma, Gallstone formation |
| **Skin** | Chronic urticaria, Schoenlein-Henoch purpura, Atopic dermatitis, angioedema, rosacea, psoriasis, alopecia areata, Sjögren Syndrome |
| **Others** | Extragastric MALT-lymphoma, Growth retardation, preeclampsia, hyperemesis gravidarum, glaucoma, oral ulcers, urethritis, inflammatory bowel diseases, glaucoma. |

*H. pylori* infections bring out a remarkable immunomodulation, which are activated by chronic inflammation (9). Chronic infection results in a mainly Th1 response, resulting in the production of IL-2 and IFN-gamma as well as other inflammatory cytokines like TNF-α, IL-6, IL-10, and IL-8. (10). This chronic infection due to *H. pylori* can also cause anarchic growth and proliferation of CD5+ B lymphocytes that produce poly- and auto-reactive IgM and IgG3 antibodies (11). Several studies have reported that Toll-like receptors (TLR) and Treg cells play roles in the immune pathogenesis of *H. pylori* infection and it is suggested in an experimental study there might be an interplay between TLR signaling and Treg cells which is significant for restricting *H. pylori* colonization and suppressing the inflammatory response (12).

*H. pylori* have an ability of immunomodulatory effect. The immunomodulatory features of the bacterium reprogram the immune system towards immunological

tolerance and help the bacteria in setting up a persistent infection (13). As a result, the products of the local immune responses could migrate to extra-gastric region and this might clarify the association between *H. pylori* infection and the diversity of extra gastric diseases, as well as autoimmune disorders (14).

In contrast, some epidemiological data suggests a protective effect of *H. pylori* infection against the development of various sicknesses with an autoimmune component. The proposed mechanism for this effect may define as *H. pylori*'s ability to induce immune tolerance and restrict inflammatory processes (15).

In view of these data, researchers have investigated the role of *H. pylori* as a pathogenic determinant for idiopathic extraintestinal diseases, in case of immune dysregulation.

This paper reviews current literature on the role of *H. pylori* infection within the pathological process of extraintestinal diseases taking into account the recent literature.

## *Rheumatoid arthritis*

Rheumatoid arthritis (RA) is an autoimmune, chronic inflammatory disorder that causes irreversible joint deformities and functional impairment. As with many other connective tissue disorders, the etiopathology of RA is not clearly understood. A report from Turkey, Yula E. et al. (2016) researched active *H. pylori* infection rate and CagA virulence marker positivity in patients with various autoimmune diseases, including RA and SLE, and they suggested that in a similar manner to the some literature their first results recommended that active *H. pylori* infection rates are higher in patients with autoimmune diseases when compared with their routine laboratory data (16). Similarly, in a recent study, El-Hewala ASI, et al. (2015) was to assess the effect of *H. pylori* treatment on disease activity in patients with rheumatoid arthritis and the authors suggested that *H. pylori* treatment may induce a significant improvement of the disease activity over two months (17). On the other hand, a study of 1815 Japanese RA patients, 49.3% were reported to have *H. pylori* antibodies, which was lower compared with the healthy Japanese subjects (18).

There are few data in the literature on this topic and the possible effect of *H. pylori* infection in the pathogenesis of RA remains controversial.

## *Parkinson's disease*

Parkinson's disease is a chronic, progressive and degenerative disorder of the central nervous system and in most people is idiopathic. Recently, some studies have advised that chronic *H. pylori* infection may worsen the neurodegenerative process in Parkinson's disease. Tan et al. (2015) reported in a large cross-sectional study showed a link between *H. pylori* and worse Parkinson's disease motor severity (19). In addition, it has been suggested that eradication of *H. pylori* infection improves levodopa action, clinical symptoms and quality of life in patients with Parkinson's disease (20). Interestingly, Blaecher C, et al. (2013) declared that frequency of *Helicobacter suis* is significantly higher in patients with idiopathic parkinsonism than healthy subjects (21).

An another exciting assumption is that in case of *H. pylori* infection is not controlled by the immune system or not eradicated, *H. pylori* may causes the development of Parkinson's disease by damaging dopaminergic cells in central nervous system. (22).

## *Multiple sclerosis*

Multiple sclerosis (MS) is a multifactorial, complex, chronic inflammatory and neurodegenerative disease of the central nervous system. Gavalas E. et al (2015), reported that *H. pylori* infection appears to be more frequent in MS patients (23). A recent report indicated the presence of immunomodulating features of "Sydney Strain-1 antigen" administration in an experimental model of MS, recommending the possible role of *H. pylori* infection in the mechanism of the disease (24). Long Y, et al. showed *H. pylori* seropositivity in patients with MS, though it did not differ considerably when compared with controls (25). In contrast, a recent meta-analysis reported that *H. pylori* infection and MS are negatively correlated, particularly in Western countries (26). These conflicting findings among the aforementioned reports may be due to ethnicity, and methodological dissimilarity.

## *Cardiovascular Disease*

Cardiovascular disease, including coronary artery disease, peripheral artery disease and stroke are the leading causes of mortality and morbidity globally. The possible effect of *H. pylori* infection in the pathogenesis of cardiovascular disorders remains controversial. Many

epidemiological researches have been performed to detect association between ischemic heart disease, lipid abnormalities and the pathogen (27). Recently, it has been reported that *H. pylori* may be present at the level of the carotid plaques. Because of the some strains elicit a strong local inflammatory response, particularly *cagA* gene positive strains; the presence of the bacteria may contribute to plaque instability and to the development of ischemic stroke (28). Despite of these studies, whether the association is still unclear. A study from Japanese population examined possible relationships between *H. pylori* infection and risk of death from coronary heart disease and stroke in a large prospective cohort study (29). They suggested that there is no link between *H. pylori* infection and coronary heart disease and stroke mortality risk. In contrast, Sagar V. et al. (2016) researched the prevalence and association of *H. pylori* infection in patients of ischemic cerebrovascular stroke and they suggested there is link between *H. pylori* infection and acute cerebral ischemia (28). But they found no considerably association between *H. pylori* seropositivity and carotid plaque instability. In a recent work from Korea, the authors investigated the relationship of current *H. pylori* infection with lipid profile and cardiovascular disease and its eradication effect (30). In a similar manner to the literature, they have declared that the current infection with the pathogen had a positive association with high LDL, low HDL, and cardiovascular disease. They also reported that successful *H. pylori* eradication decreased the risk of high LDL and low HDL. However, eradication of the bacteria did not reduce the risk of cardiovascular disease.

## *Skin diseases*

Autoimmune based dermatological pathologies are characterized by dysregulation of the immune system that causes loss of self-tolerance to dermal antigens. Many studies have been reported an association between idiopathic chronic urticaria, acne rosacea, alopecia areata and *H. pylori* infection (31-33). Treatment of *H. pylori* infection has been reported to be effective in some patients with psoriasis, Schoenlein-Henoch purpura chronic autoimmune urticaria and alopecia areata (11). Some studies declared higher prevalence of *H. pylori* infection in patients with systemic sclerosis, than in healthy individuals (34). Despite that, there is conflicting data about the association of *H. pylori* infection with scleroderma, Behçet's disease and autoimmune bullous diseases.

One of the most researched skin diseases is immune thrombocytopenic purpura (ITP). Several reports have recommended a pathological link between ITP and *H. pylori* infection. Clinical experiences have described a spontaneous resolution of ITP symptoms in approximately half of chronic ITP patients taking after treatment against *H. pylori* infection (35). Also, a randomized controlled trial suggested that, *H. pylori* eradication plays significant role in the management of *H. pylori* infected chronic ITP children and adolescents (36).

It is well known that the prevalence of eczema is increasing, particularly in developed countries where the *H. pylori* seroprevelance is relatively low. The reported risk factors linked with increased prevalence of eczema include higher level of family education, higher socioeconomic status, smaller family size and urban environment. Opposite of these conditions is known as risk factors for higher prevalence of *H. pylori* positivity. Similarly, in a recent work, Ali AM et al., reported that *H. pylori* infection is associated with childhood eczema in genetically predisposed atopic children and likewise a considerable inverse correlation between atopic dermatitis and positive *H. pylori* serology (37). In addition, a meta-analysis provides evidence that *H. pylori* infection is inversely associated with atopy (38).

Recent studies proposed a potential relationship between rosacea, psoriasis and *H. pylori*. Information from the literature involving the H. *pylori* infection in psoriasis are not clear; on the other hand, a recent study has recommended that *H. pylori* seems able to affect the clinical severity of psoriasis (39). However, several researches reported that the prevalence of *H. pylori* infection was considerably higher in patients with rosacea (40).

## *Migraine*

Migraine is a common, multifactorial, disabling, an episodic, hereditary neurovascular headache disorder (41). The well-known vascular theory of migraine is that migraine headache is caused by the dilatation of blood vessels, while the aura of migraine resulted from vasoconstriction. It has been reported that C-reactive

protein, which can increase blood-brain barrier permeability, and some pro-inflammatory cytokine levels rise in migraineurs (42, 43), and likewise it has been suggested that single nucleotide polymorphisms in TNF-α and IL-1 are associated with migraine may relevant to the etiology of the disease (44). Due to the high levels of IL-17, the cytokine most strongly associated with autoimmune disorders, it has been suspected that migraine may also be associated with autoimmune disorders (45).

Several studies presented a positive correlation between *H. pylori* infection and migraine headache. It has been proposed that the pathogenic role of the bacterium in migraine, in light of a relationship between the host immune response against the *H. pylori* and the chronic release of vasoactive substances. The proposed factors of the relationship between migraine and *H. pylori* infection included inflammation, nitric oxide imbalance, oxidative stress, or virulence of *cagA*-positive strains (46, 47). Similarly, in a recent study, it is reported that mean of *H. pylori* IgM antibody in migrainous patients showed a significant difference with a healthy control group (48). Nowadays, Mann, NS. et al. reported a meta-analysis about the possible relationship between *H. pylori* and migraine (49). They reported that 1084 cases of migraine associated with *H. pylori* and in some studies elimination of the bacteria resulted in amelioration of migraine symptoms.

## *Alzheimer's disease*

Alzheimer's disease is a neurodegenerative disease which several causes have been suggested like relationship with known pathogens. Most of the infectious hypotheses are proposed by the alteration of the blood-brain barrier and the stimulation of neuroinflammation in the central nervous system that may play a role, particularly in the decrease of amyloid peptide clearance (50,51). Some bacterial or viral pathogens have been incriminated, including *Chlamydia pneumonia*, *H. pylori* and Herpes simplex virus-1 (52).

Although, the direct laboratory evidence is lacking, *H. pylori* infection has been reported to be related to a high risk of Alzheimer's disease. In a recent study, researchers investigated the effect of *H. pylori* infection on tau phosphorylation due to abnormal hyperphosphorylation of microtubule-associated protein tau is involved in the pathogenesis of Alzheimer's disease (53). The authors, Xiu-Lian W. et al. reported evidence supporting the role of *H. pylori* infection in Alzheimer's disease-like tau pathology and they suggested that *H. pylori* eradication may be useful in the prevention of tauopathy.

## *Vitamin and mineral deficiency*

It is reported that *H. pylori* infection was associated with an enhanced rate of iron deficiency anemia, cobalamin (vitamin B12), folic acid, alpha-tocopherol, beta-carotene and vitamin C deficiency (54). One of the suspected mechanisms for these deficiencies is that *H. pylori*-induced gastritis leads to a functional inhibition of the parietal cells and causes hypochlorhydria. Thus, higher gastric pH causes iron malabsorption and also have a major role in the development of vitamin deficiencies like folate, vitamin B12 and vitamin A.

## *Diabetes mellitus*

It is suggested that *H. pylori* infection may associated with insulin resistance, diabetes mellitus and metabolic syndrome. However, the relationship between *H. pylori* infection and type 2 diabetes mellitus is controversial, as some studies revealed a higher prevalence of infection in diabetic patients while others reported there is no significant difference (55-57). Some studies from Asia have reported on an relationship between *H. pylori* infection with insulin resistance in normal-weight individuals. Recently, Nasif WA. et al. (2016), reported that infection with *H. pylori* in type 2 diabetes mellitus was higher when compared to non-diabetic population and seems no link with glycemic control (58); Likewise, they proposed that diabetes seems to be associated with increased oxidative stress in *H. pylori* infection and they reported that significantly raised serum Oxidized low-density lipoprotein (Ox-LDL) levels in diabetes patients with positive *H. pylori* infection, proposing hypothesis that high serum level of Ox-LDL levels in diabetes patients with positive *H. pylori* infection considered as a risk factor for atherosclerotic vascular disease. One recent study has shown obese patients do not provide evidence for an enhanced insulin resistance state associated with gastric *H. pylori* infection, but they suggested that the presence of the bacterium in gastric biopsies is associated with an adverse lipid profile (59). In addition, it was suggested that eradication rate of *H.*

*pylori* is significantly lower in patients with obese non-diabetic than healthy subjects (60).

### *Oral pathologies and Sjögren Syndrome*

Several studies indicated that *H. pylori* can be isolated from the oral cavity, salivary secretions and dental plaque. The presence of the pathogen in some oral lesions like burning, halitosis and lingual dorsum hyperplasia has been reported with high frequency (61). Alireza Monsef Esfahani et al. (2015) reported that *H. pylori* infection plays important role in the pathogenesis of Sjögren Syndrome a chronic autoimmune disease characterized by lymphocytic infiltration of exocrine glands (62).

Recurrent aphthous stomatitis is one of the common oral mucosal diseases with unknown etiology. Gülseren D. et al. (2016) researched possible link between recurrent aphthous stomatitis, and periodontal disease and *H. pylori* infection in a cross-sectional study and they suggested that *H. pylori* might have played an etiological role in recurrent aphthous stomatitis and might have caused periodontal disease and eradicating *H. pylori* may be useful to prevent the disease (63).

### *Obesity*

In developed countries, the prevalence of overweight and obese individuals has substantially increased, but the prevalence of *H. pylori* has decreased. It has been speculated that decreasing prevalence of *H. pylori* might represent a risk or contributing factor to the endemic of obesity in western countries. But, the relationship between gastric *H. pylori* infection and body mass index (BMI) is controversial. While several cross-sectional studies have reported a link between *H. pylori* infection with BMI, others did not find an association (64). Arslan E, et al. has shown an increased prevalence of gastric *H. pylori* infection in obese individuals when compared to normal-weight counterparts (65). Interestingly, Lender N. et al., reported that the prevalence of gastric *H. pylori* colonization in various countries is inversely related to the prevalence of obesity (64).

### *Bronchial asthma and chronic obstructive pulmonary disease*

Chronic obstructive pulmonary disease (COPD) is considered the fourth leading cause of death worldwide. Controversial results of *H. pylori* seroprevalence have been achieved in patients with bronchial asthma, sarcoidosis, pulmonary tuberculosis, cystic fibrosis, chronic bronchitis and lung cancer (66). For example, a recent systematic review has reported that there is a relationship between *H. pylori* infection and extra-gastric diseases like bronchiectasis, asthma, COPD, lung cancer, and lung tuberculosis (67). It is well known that *H. pylori* prevalence in developed countries has been declining simultaneously with increases in childhood asthma and other allergic diseases. Thus, several studies have linked these phenomena. Lim JH et al. (2016) have declared an inverse association between *H. pylori* infection and asthma among young adult, and they proposed that the underlying immune mechanism induced by *H. pylori* infection may affect allergic reactions associated with asthma in young adults due to its low prevalence (15). Also, they supposed that, *H. pylori* infection may inhibit development of asthma in some way in young adults due to effects on the immune system. On the other hand, den Hollander WJ et al. reported that colonization of a European child with a CagA negative strains at age 6 was associated with an increased prevalence of asthma, but they declared no link for non-European children (68). We think that the underlying mechanisms for the relationship between asthma and *H. pylori* infection requires further research like the other diseases.

### *Inflammatory bowel diseases (Crohn's disease, ulcerative colitis)*

Interestingly, some epidemiological data suggest a protecting effect of *H. pylori* infection against the development of autoimmune diseases and, additionally, there are laboratory data illustrating *H. pylori*'s ability to induce immune tolerance and limit inflammatory responses (69). Inflammatory bowel disease is an important growing health problem, globally. In last decades, a lot of developing countries have experienced a spectacular climb in the incidence of the disease. Recently, a meta-analysis indicated a significant negative link between *H. pylori* infection and inflammatory bowel diseases that supports a possible protective profit of *H. pylori* infection against the development of the disease (69). The researchers also reported that further prospective studies determining the role of *H. pylori* and its eradication in the evolvement of inflammatory bowel

diseases are required by taking into account the role of confounders like environmental factors.

## Conclusion

The distinctive ability of *H. pylori* to inveterately infect the gastric tissue to activate inflammation and host immunological response recommends its role in various autoimmune diseases. Although there are conflicting and controversy data in some diseases, in the light of mentioned reports, it is currently accepted; that the presence or absence of *H. pylori* infection might influence the chance of developing of many autoimmune diseases. Despite extensive medical advancement many questions still remain unanswered and, further studies analyzing the supposed causality of the observed relationship between *H. pylori* infection and extra-intestinal diseases are clearly in need. We think that, if such causality is confirmed, this could have a great effect on clinical practice as it will probably goes to the recommendation of *H. pylori* screening and eradication in various diseases as a clinical standard therapy.

Although, lots amount of studies are required to address the role of *H. pylori* in pathogenesis of various autoimmune diseases, some reports give a hope that eradication of the bacteria could be a cure or to reduce the severity of some diseases.

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