

Positive Effect of *Helicobacter pylori* Treatment on Outcome of Patients With Chronic Spontaneous Urticaria

A Randomized Double-Blind Pilot Study

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ABSTRACT

Objectives: The association between *Helicobacter pylori* and chronic spontaneous urticaria (CSU) is controversial. Therefore, we aimed to directly diagnose *H pylori* by polymerase chain reaction (PCR) in gastric tissue from patients with CSU and to investigate the association between *H pylori* eradication therapy and CSU remission.

Methods: Twenty-seven of 72 patients with CSU who were positive for *H pylori* stool antigen and PCR in gastric biopsy specimens were randomized to receive either anti-*H pylori* treatment or placebo.

Results: Patients with *H pylori* were found to have significantly lower hemoglobin concentrations with microcytic hypochromic anemia and a significantly higher occurrence of dyspepsia symptoms. All *H pylori*-treated patients (except two) showed significant improvement of the urticaria itching and red wheals after 2 weeks of therapy compared with the placebo group ($P < .001$). The response rate to treatment was 85.7% (12 patients; 95% confidence interval, 64.3%-100.0%). The two patients who failed to eradicate *H pylori* had an *H pylori* strain resistant to amoxicillin.

Conclusions: An association was observed between CSU and presence of *H pylori* infection in the gastric tissue. Whether this is a causal relationship or not remains to be discovered, but treatment of *H pylori* can significantly improve the symptoms of CSU.

Key Points

- The association between *Helicobacter pylori* and chronic spontaneous urticaria (CSU) is controversial.
- We address direct detection of *H pylori* by polymerase chain reaction in gastric tissue and a study of the effect of eradication therapy on urticaria course and the effect of resistant *H pylori* on the persistence of urticaria symptoms.
- Testing for *H pylori* should be included in the diagnostic workup of CSU, especially in patients with no response to standard chronic urticaria treatment or those with concurrent dyspeptic complaints. Detection of resistant *H pylori* strains before start of therapy is of importance.

Patients with chronic urticaria have chronic hives, itching, and erythematous wheals and usually have flares that come and go for months to years. Diagnosis is mainly clinical, and only a limited nonspecific laboratory workup should be considered. The mainstay of treatment is to avoid the triggers, if identified, and the first-line pharmacotherapy is second-generation H1 antihistamines.¹

Chronic spontaneous urticaria (CSU) (formerly known as chronic idiopathic urticaria) represents 80% to 90% of the cases of chronic urticaria, which is associated with depression and anxiety disorders and affects patients' quality of life. Urticaria pathogenesis is either by immunoglobulin E (IgE)-mediated or non-IgE-mediated release of histamine and other inflammatory mediators from mast cells and basophils. Some infectious agents can trigger urticaria through direct mast cell degranulation. CSU may be related to an underlying autoimmune mechanism.²

Helicobacter pylori is a gram-negative bacterium that persistently colonizes the stomach, and its presence is associated with an increased risk of peptic ulcer and gastric cancer.³

H pylori infection is the most prevalent bacterial infection globally, with variations in its prevalence between developed and developing countries. *H pylori* prevalence in Egypt ranges in children from 13% to 72% and in adults from 26% to 90% and is more prevalent in rural areas and among those of low socioeconomic status.⁴

The association between *H pylori* and spontaneous urticaria is subject to much dispute. Several studies supported a lack of relationship between *H pylori* infection and CSU,^{5,6} and other studies found no correlation between *H pylori* eradication and remission of urticarial symptoms, although the prevalence of *H pylori* infection was high in CSU.⁷⁻⁹

H pylori-infected gastric mucosa is a rich compartment of T-regulatory cells, which may influence systemic immunity and thus may play a role in CSU pathogenesis. Gastric epithelial cells (which act as antigen-presenting cells) are induced by *H pylori*, leading to expression of immune checkpoint receptors that sway T-lymphocyte function and circumvent effective T-effector lymphocyte responses.¹⁰ Therefore, *H pylori* may protect against asthma and related disorders, or it may be a marker for other changes in microecology that are the protective factors.¹¹

H pylori may play a role in CSU pathogenesis by induction of immunologic reactions to *H pylori* antigens or deregulation of T lymphocytes in the gastric tissue, leading to release of multiple cytokines, which may induce immunologic reactions, including CSU,^{12,13} by the following proposed mechanisms: *H pylori* may increase the stomach-lining permeability, increasing the exposure to allergens in the gastrointestinal tract. Also, the immune response to *H pylori* yields antibodies that stimulate histamine release in the skin. Finally, patients with urticaria develop specific IgE against *H pylori* through IgE-containing cells in the gastrointestinal tract.^{12,13}

We conducted a randomized pilot study to investigate the association between *H pylori* and CSU.

Materials and Methods

This was a randomized, double-blind, placebo-controlled pilot study. In total, 214 new patients with urticaria referred to the dermatology department and the tropical medicine and infectious diseases department in Tanta University Hospital from January 2018 to January 2020 were eligible for participation in this study.

We included patients with newly diagnosed CSU. We excluded patients with a contraindication or an allergy to the drugs used in this study as well as those taking proton pump inhibitors or antibiotics in the 6 weeks prior to the trial. We excluded patients with allergy history for food, environmental factors, and drugs. Patients with chronic diseases such as liver cirrhosis, renal impairment, and gastrointestinal malignancies as well as patients with physical urticaria or urticaria vasculitis, or those receiving any antihistamines or corticosteroids within the 7 days preceding the study, were also excluded.

Diagnosis of CSU was based on history and clinical symptoms for more than 6 weeks with urticaria lesions visible 3 days or more per week. Routine investigations including CBC, erythrocyte sedimentation rate, C-reactive protein, antinuclear antibodies, thyroid functions, blood sugar, urea, creatinine, and liver functions tests were performed.

A full medical history was taken from all patients. Clinical and demographic data were recorded.

Esophagogastroscope was performed for patients with positive *H pylori* Ag in stool. Four gastric biopsy specimens were obtained from each patient: two from the antrum and two from the gastric corpus for *H pylori* DNA detection by polymerase chain reaction (PCR) as well as for detection of clarithromycin- and amoxicillin-resistant strains in those patients who had *H pylori* eradication failure. The gastric biopsy specimens were stored in sterile saline and kept at -70°C until processed. Patients with a positive stool Ag test were considered infected with *H pylori*, and confirmation was by detection of 16S ribosomal RNA (rRNA) in gastric biopsy specimens.

- DNA extraction from gastric biopsy specimens and PCR assays. DNA was extracted using the QIAamp tissue kit (QIAamp DNA Mini Kit; QIAGEN), following manufacturer guidelines.
- Detection of *H pylori* 16S rRNA gene. Conventional PCR assay was done in a volume of 50 μL with approximately 5 mg extracted DNA, 200 mmol/L (each) dNTPs, 25 pmol for each primer, 1 U Taq polymerase (Gotaq Flexi DNA, M8305; Promega), and 1.5 mmol/L magnesium chloride in PCR buffer using primers. Denaturation and annealing followed by final extension were done to obtain a 110–base pair (bp) amplicon (Figure 1).^{14,15}
- Detection of Pbp1A gene conferring amoxicillin resistance. Conventional PCR assay was done in a volume of 50 μL , with about 1 mg extracted DNA, 0.5 U Pfu DNA polymerase (Thermo), and 200 mmol/L (each) dNTPs in a PCR 5-mL Pfu buffer with magnesium sulfate and 25 pmol primer. Denaturation and annealing followed by final extension were done to obtain a 2,300-bp amplicon.

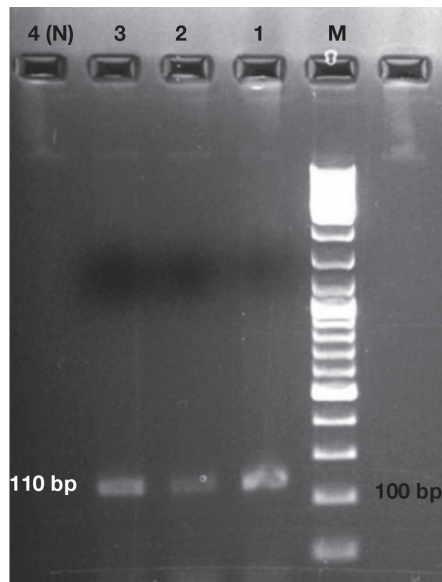


Figure 1 *Helicobacter pylori* 16S ribosomal RNA polymerase chain reaction products by agarose gel electrophoresis. The 110–base pair (bp) fragment was detected in samples 1 to 3 (lanes 1 to 3). M, molecular weight marker (ladder 50 bp). Lane N, negative control.

The PCR product was separated on 2% agarose gel with ethidium bromide and a 100-bp ladder as DNA molecular weight standard. A negative control was included. PCR products were assessed under UV light.^{14,16,17}

- Sequencing of DNA fragments of Pbp1A genes. DNA fragments were purified from agarose gel using the Thermo Scientific GeneJET Gel Extraction Kit, according to the manufacturer's instructions. DNA sequencing was done by the Sanger sequencing method following the manufacturer's guidance.^{14,17}
- Detection of 23S rRNA gene conferring clarithromycin resistance. Real-time (TaqMan) PCR assay was done in a volume of 25 μ L, with about 10 ng purified DNA, 36 μ L of each primer, 12.5 mL universal PCR master mix (ABI), and 8 mmol/L FAM- and VIC-labeled TaqMan MGB probes.¹⁸ Primers and probe sequences were used according to Lins et al¹⁹ for detection of DNA wild type, mutation 23S rRNA A2142G, and mutation 23S rRNA A2143G. Real-time PCR amplification was done at 95°C for 10 minutes and 40 cycles of 92°C for 15 seconds and 60°C for 1 minute.^{23,28}

Testing for *H pylori* Antigen in the Stool

Sandwich technique enzyme-linked immunosorbent assay (ELISA) kit (catalog No. HPY35-k01; Eagle

Biosciences) was used to measure *H pylori* Ag in a fresh 1- to 2-mL liquid stool sample or a 1- to 2-g solid sample, and the color change was measured spectrophotometrically at a wavelength of 450 nm.

Randomization of *H pylori* Stool Ag and PCR-Positive Patients With CSU

Patients with CSU who proved positive for *H pylori* infection were randomized into two groups using a computerized random-number generator to select randomly permuted blocks and an equal allocation ratio. Two authors (M.E. and E.S.E.S.) recruited and enrolled participants. Both investigators and patients were blinded to the treatment administered. The two groups were matched regarding age, sex, and basic and clinical characteristics. In the *H. pylori* treatment group, patients received triple anti-*H pylori* treatment, including clarithromycin 500 mg twice daily, amoxicillin 1 g twice daily, and omeprazole 40 mg twice daily for 2 weeks. In the placebo group, patients received placebo for 2 weeks. Both groups received antihistaminic agents daily. The study was performed according to the Declaration of Helsinki and the Ethics Committee of the Faculty of Medicine, Tanta University, and an informed consent was signed by every patient before enrollment in the study.

Assessments

All patients were followed weekly during the period of treatment. Patients' contact numbers were taken, and patients were called twice weekly and asked about the frequency and severity of itching and any side effects of treatment. The urticaria activity score (UAS7) was calculated for each patient. UAS7 is a validated scoring system for chronic urticaria, calculated as the sum of the daily number of hives score and the itch severity score over 7 days. UAS7 values range from 0 to 42, with higher values reflecting higher disease activity.² Testing for *H pylori* antigen in stool was done at weeks 4 and 8 following the end of therapy to assess cure.

Outcomes

The primary outcome of the trial was the number of patients with CSU who achieved remission of urticaria according to the UAS7 score at the end of the second week of *H pylori* treatment and maintained remission at 4 and 8 weeks following the end of therapy. The secondary outcome was the prevalence of *H pylori* in patients newly diagnosed with CSU.

Statistical Analysis

Data were analyzed with SPSS statistical package (version 20; SPSS). The Student *t* test was performed

to compare quantitative variables between two groups of normally distributed data. The χ^2 test was performed to examine the association between qualitative variables (including the UAS7 score) with the z test to compare column proportions. Fisher exact test with Yates correction was used when cells were fewer than five. A two-sided P value of less than .05 was considered statistically significant.

Results

Of the 214 patients screened for study participation, 142 were excluded (116 failed to fulfill the inclusion criteria, 10 fulfilled the exclusion criteria, and 16 declined to participate). Thus, 72 patients with newly diagnosed CSU were enrolled in this study (Figure 2).

Among the 72 patients with CSU, 28 (38.9%) were men and 44 (61.1%) were women. Their mean age was 35.63 ± 12.42 years (range, 20–75 years). Two (2.8%) patients were smokers. Dyspepsia symptoms were present in 34 (47.2%) patients (Table 1).

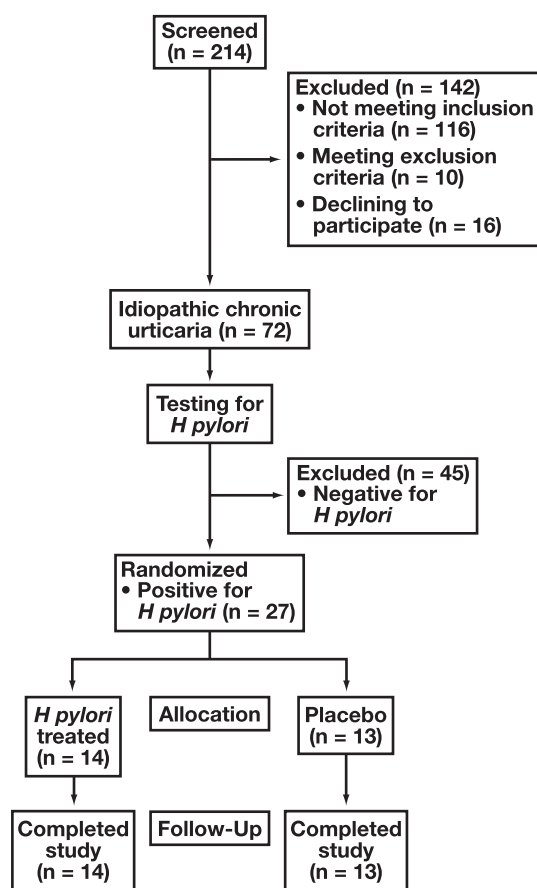


Figure 2 Study analysis population.

Table 1

Chronic Spontaneous Urticaria Patient Characteristics^a

Variable	<i>Helicobacter pylori</i> Negative (n = 45)	<i>Helicobacter pylori</i> Positive (n = 27)	P Value
Age, mean $\pm$ SD, y	28.16 $\pm$ 9.15	30.85 $\pm$ 7.92	.473
Hemoglobin level, mean $\pm$ SD, g/dL	12.41 $\pm$ 2.81	10.18 $\pm$ 2.94	.031
Sex			
Male	16 (35.6)	12 (44.4)	.454
Female	29 (64.4)	15 (55.6)	
Smokers	1 (2.2)	1 (3.7)	.760
Comorbidities			
Absent	39 (86.6)	23 (85.2)	.318
Diabetes mellitus	2 (4.4)	2 (7.4)	
Hypertension	2 (4.4)	0 (0.0)	
Diabetes mellitus and hypertension	2 (4.4)	2 (7.4)	
Dyspepsia symptoms	22 (48.9)	27 (100)	.009

^aValues are presented as number (%) unless otherwise indicated.

All patients with CSU were tested for *H pylori*, and 27 (39.7%; 95% confidence interval [CI], 27.9%–51.5%) proved positive. No significant differences were noted regarding age, sex, current smoking, or comorbidities, but dyspeptic symptoms were significantly higher among the *H pylori*–positive patients.

Laboratory investigations of the studied groups showed that patients with *H pylori* had significantly lower hemoglobin concentrations and microcytic hypochromic anemia ($P = .031$).

All patients with CSU with *H pylori* included in the study had dyspeptic complaints, and therefore gastroscopy was performed. Various endoscopic findings were noted, ranging from mild gastritis to peptic ulcer. Four biopsy specimens were obtained from each patient, two from the gastric antrum and two from the gastric corpus for *H pylori* detection by PCR.

The active treatment and placebo groups were matched for age and sex. There was no significant difference between them regarding current smoking status, comorbidities, or dyspepsia symptoms.

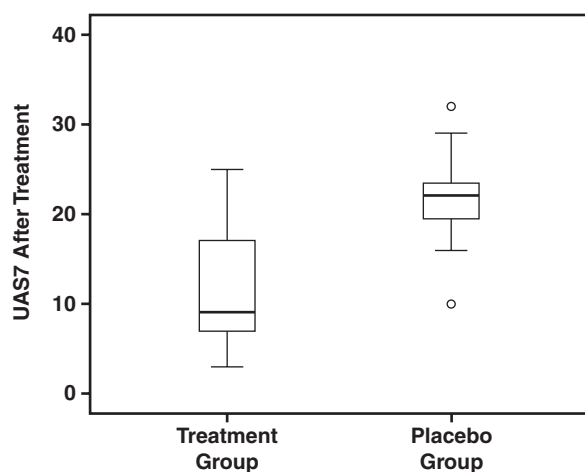
In the *H pylori*–treated group (except two patients), urticaria itching and red wheals significantly decreased after 2 weeks of therapy compared with the placebo group ($P < .001$). There was no significant change of symptoms following treatment with placebo. There was no significant difference between the groups regarding UAS7 before treatment ($P = .830$). However, on comparing UAS7 scores before and after treatment, the placebo group did not show any significant difference ($P = .157$), while the treatment group showed a highly significant difference ($P = .001$)

(Table 2) and Figure 3).

Table 2**Comparison Between the Treatment Group and Placebo Group of *Helicobacter pylori*-Positive CSU Patients^a**

Variable	Treatment Group (n = 14)	Placebo Group (n = 13)	P Value
Age, mean $\pm$ SD, y	30.85 $\pm$ 7.92	28.16 $\pm$ 9.15	.473
Sex			
Male	6 (42.9)	6 (46.2)	.863
Female	8 (57.1)	7 (53.8)	
Smokers	0 (0.0)	1 (7.7)	.290
Comorbidities			
Absent	11 (78.6)	12 (92.3)	.366
Diabetes mellitus	1 (7.1)	1 (7.7)	
Diabetes mellitus and hypertension	2 (14.3)	0 (0.0)	
Dyspepsia symptoms	14 (100.0)	13 (100.0)	—
Response to treatment			
No improvement	2 (14.3)	13 (100.0)	<.001
Improvement	12 (85.7)	0 (0.0)	
UAS7 before treatment			
Mild CSU (7-15)	1 (7.1)	1 (7.7)	.830
Moderate CSU (16-27)	10 (71.4)	8 (61.5)	
Severe CSU (28-42)	3 (21.4)	4 (30.8)	
UAS7 after treatment			
Well-controlled CSU (1-6)	3 (21.4)	0 (0.0)	.001
Mild CSU (7-15) ^b	8 (57.1)	1 (7.7)	
Moderate CSU (16-27)	3 (21.4)	10 (70.9) ^b	
Severe CSU (28-42)	0 (0.0)	2 (15.4)	

CSU, chronic spontaneous urticaria; UAS7, urticaria activity score.

^aValues are presented as number (%) unless otherwise indicated.^bSignificantly higher than their corresponding category in the other group.**Figure 3** Box plot of urticaria activity score (UAS7) after treatment in the studied group.

The regimen was well tolerated by all patients, and mild side effects were reported in one patient who had nausea, which was self-limited.

Twelve patients in the treated group had negative *H pylori* stool antigen test results at 4 and 8 weeks after the end of therapy. The response rate to treatment in this group was 85.7% (12 patients; 95% CI, 64.3%-100.0%).

Two patients showed failure to eradicate *H pylori* with persistence of dyspeptic symptoms and positive *H pylori* Ag in stool. Gastroscopy was done, and biopsy specimens were taken to test for clarithromycin- and amoxicillin-resistant strains of *H pylori*. Both proved to have *H pylori* amoxicillin-resistant strains.

Discussion

The association between *H pylori* and CSU is subject to much dispute. For this reason, we conducted a randomized, double-blind, placebo-controlled pilot study to investigate the association between *H pylori* and CSU and study the effect of *H pylori* eradication therapy on CSU remission.

Among the 214 patients screened for study participation, 72 (33.6%) with newly diagnosed CSU were enrolled in this study.

Studies on chronic urticaria in Egypt have revealed that patients with chronic urticaria represent 1.13% of the total number of patients who attend the dermatology clinics per year and up to 1% of the general population.^{20,21}

In Egypt, multiple studies on the epidemiology of *H pylori* showed that the overall prevalence is 72.38% to 88%, and the main risk factor is overcrowding and residence in socioeconomic-deprived areas with a higher burden of *H pylori* infection in rural areas than in urban areas.^{22,23} This high prevalence is why we did not use ELISA in the present study as it cannot differentiate between old and new infections. Urea breath test is not widely available, requires specialized equipment for CO₂ measurement and infrastructure for radioactive materials management, and is an expensive test.

Yoshimasu and Furukawa²⁴ in 2014 found that the positive rate of anti-*H pylori* IgG antibodies was 47.7% in all patients with CSU. The study by Mogaddam et al²⁵ in 2015 found that 36% of patients with CSU were infected with *H pylori*.

In the *H pylori*-treated group of the current study, the response rate was 85.7% (12 patients; 95% CI, 64.3%-100.0%). Our results are in agreement with Chiu et al,²⁶ who found complete remission of urticaria in 63.6% of patients and concluded that *H pylori* may play a role in the development and disease course of CSU. Another study in 2008²⁷ found that 72.05% of patients with CSU responded to *H pylori* eradication therapy.

On the other side, a study in 2000⁵ and Howden in 1999⁶ supported a lack of relationship between *H pylori* infection and the CSU. Several studies found no correlation between *H pylori* eradication and remission of urticaria symptoms, although the prevalence of *H pylori* infection was high in CSU.⁷⁻⁹

These studies were open rather than blinded and were done without a placebo control. There are other causes of heterogeneity between the studies, including age, time period, modality of testing, and different methods and assays for the diagnosis of *H pylori*, with different sensitivities and specificities. Many studies were depending on detection of *H pylori* by the old ordinary methods such as serology, which has a high false-negative rate and cannot differentiate between old and new infections, and two drugs used for its eradication for a short duration, which are not enough to eradicate the infection. On the other side, we used in our study the highly sensitive PCR technique for *H pylori* detection and used three drugs for 14 days, which may be the reason for the high remission rate plus geological differences in these studies from ours.

The epidemiology of *H pylori* infection has changed with improvements in sanitation and methods of eradication, and it is possible that different infectious agents may contribute to the pathogenesis of CSU in different geographic areas.

In this study, two (14.2%) of 14 patients showed failure of treatment, and this was due to *H pylori* strain resistant to amoxicillin. Two recent studies were conducted in Egypt in 2016 and 2018 to assess antimicrobial resistance rates of *H pylori* strains recovered from patients who underwent upper endoscopic examination. Amoxicillin resistance among the studied *H pylori* strains was 18.3%, and 20.8% respectively.^{14,28}

Limitations of the study are the small number of the patients, as it was a single-center study conducted during a limited time period and that we did not have a long-term follow-up of the patients for recurrence.

In conclusion, there was an association between CSU and presence of *H pylori* in the gastric tissue. Whether this is a causal relationship remains to be discovered. Testing for *H pylori* should be included in the diagnostic workup of CSU, especially in patients with no response to the customary chronic urticaria treatment or those with concurrent dyspeptic complaints.

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References

- Schaefer P. Acute and chronic urticaria: evaluation and treatment. *Am Fam Physician*. 2017;95:717-724.
- Zuberbier T, Aberer W, Asero R, et al. The EAACI/GA²LEN/EDF/WAO guideline for the definition, classification, diagnosis and management of urticaria. *Allergy*. 2018;73:1393-1414.
- Nomura A, Stemmermann GN, Chyou PH, et al. *Helicobacter pylori* infection and the risk for duodenal and gastric ulceration. *Ann Intern Med*. 1994;120:977-981.
- Alboraie M, Elhossary W, Aly OA, et al; Special Interest Group; Egyptian Association for Study of Gastrointestinal Diseases and Liver (EASGLD). Egyptian recommendations for management of *Helicobacter pylori* infection: 2018 report. *Arab J Gastroenterol*. 2019;20:175-179.
- Daudén E, Jiménez-Alonso I, García-Díez A. *Helicobacter pylori* and idiopathic chronic urticaria. *Int J Dermatol*. 2000;39:446-452.
- Howden CW. No evidence for an association between *H. pylori* and idiopathic chronic urticaria. *Dig Dis Sci*. 1999;44:485-486.
- Becker H, Meyer M, Paul E. Remission ratio of chronic urticaria spontaneous healing or result of eradication of *Helicobacter pylori* infection? *Hautarzt*. 1998;49:907-911.
- Schnyder B, Helbling A, Pichler WJ. Chronic idiopathic urticaria: natural course and association with *Helicobacter pylori* infection. *Int Arch Allergy Immunol*. 1999;119:60-63.
- Schrutka-Koebl CH, Wasilewicz-Stephani G, Gschwandler M, et al. Has eradication therapy an effect in *Helicobacter*-positive patients with chronic urticaria? *Am J Gastroenterol*. 1998;93:2632-2633.
- Reyes VE, Peniche AG. *Helicobacter pylori* deregulates T and B cell signaling to trigger immune evasion. *Curr Top Microbiol Immunol*. 2019;421:229-265.
- Arnold IC, Dehzad N, Reuter S, et al. *Helicobacter pylori* infection prevents allergic asthma in mouse models through the induction of regulatory T cells. *J Clin Invest*. 2011;121:3088-3093.
- Hernando-Harder AC, Bookan N, Goerdts S, et al. *Helicobacter pylori* infection and dermatologic diseases. *Eur J Dermatol*. 2009;19:431-444.
- Buhner S, Reese I, Kuehl F, et al. Pseudoallergic reactions in chronic urticaria are associated with altered gastroduodenal permeability. *Allergy*. 2004;59:1118-1123.
- Diab M, El-Shenawy A, El-Ghannam M, et al. Detection of antimicrobial resistance genes of *Helicobacter pylori* strains to clarithromycin, metronidazole, amoxicillin and tetracycline among Egyptian patients. *Egypt J Med Hum Genet*. 2018;19:417-423.
- Chisholm SA, Owen RJ, Teare EL, et al. PCR-based diagnosis of *Helicobacter pylori* infection and real-time determination of clarithromycin resistance directly from human gastric biopsy samples. *J Clin Microbiol*. 2001;39:1217-1220.
- Rimbara E, Noguchi N, Kawai T, et al. Mutations in penicillin-binding proteins 1, 2 and 3 are responsible for amoxicillin resistance in *Helicobacter pylori*. *J Antimicrob Chemother*. 2008;61:995-998.
- Sanger F, Coulson AR. A rapid method for determining sequences in DNA by primed synthesis with DNA polymerase. *J Mol Biol*. 1975;94:441-448.
- de Francesco V, Margiotta M, Zullo A, et al. Primary clarithromycin resistance in Italy assessed on *Helicobacter pylori* DNA sequences by TaqMan real-time polymerase chain reaction. *Aliment Pharmacol Ther*. 2006;23:429-435.
- Lins AK, Lima RA, Magalhães M. Clarithromycin-resistant *Helicobacter pylori* in Recife, Brazil, directly identified from gastric biopsies by polymerase chain reaction. *Arq Gastroenterol*. 2010;47:379-382.

20. El-Sharkawy REE, Abd-Elmaged WM, Ahmed DA, et al. Pattern of chronic urticaria and value of autologous serum skin test in Sohag Province, Upper Egypt. *Electron Physician*. 2018;10:6781-6788.
21. Abd El-Azim M, Abd El-Azim S. Chronic autoimmune urticaria: frequency and association with immunological markers. *J Investig Allergol Clin Immunol*. 2011;21:546-550.
22. Mohammad MA, Hussein L, Coward A, et al. Prevalence of *Helicobacter pylori* infection among Egyptian children: impact of social background and effect on growth. *Public Health Nutr*. 2008;11:230-236.
23. Salem OE, Youssri AH, Mohammad ON. The prevalence of *H. pylori* antibodies in asymptomatic young Egyptian persons. *J Egypt Public Health Assoc*. 1993;68:333-352.
24. Yoshimasu T, Furukawa F. Eradication therapy for urticaria with high titers of anti *H. pylori* IgG antibody. *Allergol Int*. 2014;63:37-40.
25. Mogaddam MR, Yazdanbod A, Ardabili NS, et al. Relationship between *Helicobacter pylori* and idiopathic chronic urticaria: effectiveness of *Helicobacter pylori* eradication. *Postepy Dermatol Alergol*. 2015;32:15-20.
26. Chiu YC, Tai WC, Chuah SK, et al. The clinical correlations of *Helicobacter pylori* virulence factors and chronic spontaneous urticaria. *Gastroenterol Res Pract*. 2013;2013:436727.
27. Yadav MK, Rishi JP, Nijawan S. Chronic urticaria and *Helicobacter pylori*. *Indian J Med Sci*. 2008;62:157-162.
28. Zaki ME, Sherif DM, Ali MA, et al. Molecular study of primary clarithromycin resistant *Helicobacter pylori* strains from Egyptian centre. *Int J Curr Microbiol Appl Sci*. 2016;5:165-173.