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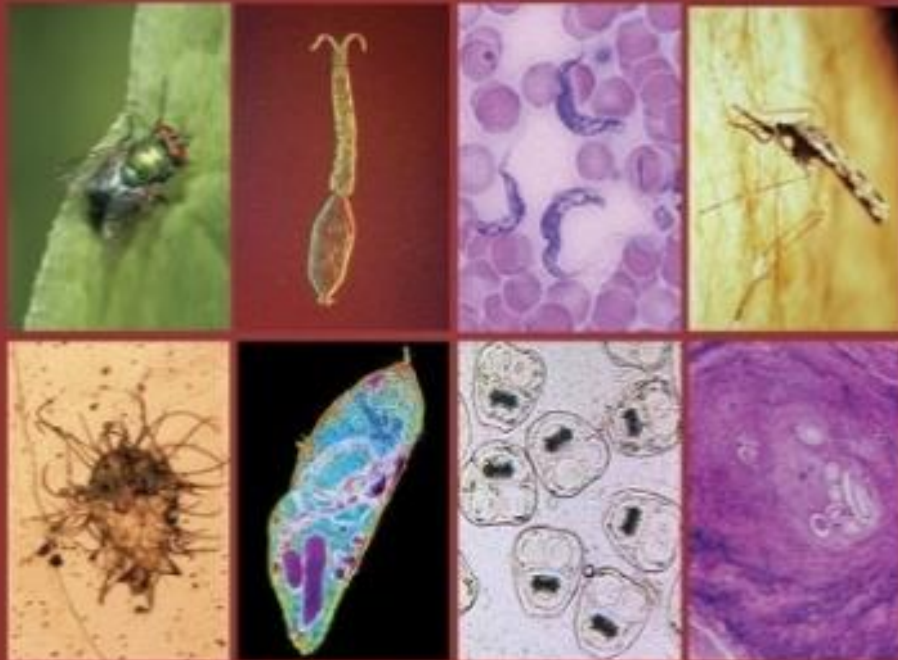

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Assessment of *Helicobacter pylori* Antigen and Enteric Parasites Among Patients with Chronic Spontaneous Urticaria

Shimaa Mohammed Abdel Aal[1]*, Amira R. Ismail[1], Amira Elbendary[2], Mousa A.M. Ismail[1], Fatma M. A. Eissa[1]

[1]Department of Medical Parasitology, Faculty of Medicine, Cairo University, Egypt.
[2]Department of Dermatology, Faculty of Medicine, Cairo University, Egypt.

***Email :** Shimaa.abdo@kasralainy.edu.eg

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ABSTRACT

Chronic spontaneous urticaria (CSU) may be associated with intestinal parasites and *Helicobacter pylori* (*H. pylori*) infection, the objective of this study was to detect intestinal parasites and *Helicobacter pylori* coproantigen in 40 patients complaining of urticaria for unknown reasons for more than 6 weeks as cases in comparison with 40 healthy controls. Intestinal parasites were detected in 17/40 (42.5%) and 6/40 (15%) cases and controls, respectively. *Blastocystis* spp. was found 14/40 (35%), and 6/40 (15%) in cases and controls, respectively which was statistical significance (p=0.039). *Cryptosporidium* spp. was found in 4/40 (10%) and *Entamoeba* spp. 2/40 (5%) in cases which were statistically non-significance.

Helicobacter pylori coproantigen was found in 24/40 (60%) cases and 14/40 (35%) in controls showing a significant association (P=0.025). The Restriction Fragment Length Polymorphism (REFLP) identified *Blastocystis* spp. in the cases after nested PCR of SSU rDNA sequences with the predominance of ST3. The 2 cases of *Entamoeba* were differentiated using multiplex PCR and found that one case was *Entamoeba dispar* and the other one was *Entamoeba histolytica.* Out of 40 cases, 52.5% reported gastrointestinal symptoms as; upper abdominal pain 25%, diarrhea 27.5% and other manifestations were reported as; fever 25% and headache 32.5%. Among the *H. pylori-positive* samples; 12/24 (50%) were co-infected with intestinal parasites; *Blastocystis spp.* 9/24 (37.5/%), *Cryptosporidium* spp. 3/24 (12.5%) and *Entamoeba dispar* 1/24 (4.2%). Non-significant differences were noticed among cases in terms of *H. Pylori* positivity regarding age, sex and clinical manifestation.

INTRODUCTION

Skin urticaria refers to the appearance of restricted or extensive red swollen papules on the skin that can last for several weeks. Angioedema may also accompany urticaria, illustrated by abnormal alterations in the subcutaneous tissue and the depth of the dermis (Kayiran and Akdeniz, 2019). Chronic urticaria (CU) is described as intermittent itching and urticaria, with or without angioedema, it may last for six weeks or more. It is categorized into urticaria which is elicited by particular stimulants or chronic spontaneous urticaria (CSU) which is provoked without stimulants (Vezir *et al.,* 2019). So, CSU elicited with unknown cause is associated with wheals, angioedema, or both lasting over six weeks. Infections for example infection with intestinal parasites, food intolerance, and autoimmunity, are assumed to be the main triggers of CSU (Vezir *et al.,* 2019, Viñas *et al.,* 2020, Fakhar *et al.,* 2021).

Enteric parasites are assumed to be a problem affecting health, particularly in undeveloped nations. Overcrowded conditions, lack of hygiene, and low socioeconomic conditions raise the possibility of acquiring enteric parasites (Vezir *et al.,* 2019). Highly dominant intestinal helminth and intestinal protozoa such as *Blastocystis* spp., have been recorded (Hernández *et al.,* 2019). *Blastocystis* spp. has been related to urticaria, as it is found in people with no symptoms in the digestive tract (Lepczyńska *et al.,* 2017). This intestinal parasite is commonly detected in human feces samples, but its medical significance and pathogenicity are still debated (Paboriboune *et al.,* 2014).

Helicobacter pylori (*H. pylori*) is a commonly found stomach microorganism because of its effect in developing chronic gastroenteritis and peptic ulcers which may predispose to cancer in the stomach. *H. pylori* is thought to be present in half of the world's population, predominantly in underdeveloped nations (Seid *et al.,* 2018). Though the actual route of transmission isn't identified exactly, some scientists have recounted that the infection can happen throughout the oral and fecal-oral route between humans (Brown, 2000, Moreira *et al.,* 2005, and Urgas and Miman, 2013). Because of doubts about ignorance of the method of infection with *H. pylori,* it has uncontrolled spread, and a high incidence (Moreira *et al.,* 2005). Numerous researchers reported that *H.Pylori* infection has been associated with chronic urticaria lesions (Abdelaziz *et al.,* 2021, and Guo *et al.,* 2021). Enteric parasites and *H. pylori* infections occur together proving the relation to each other and shouldn't be ignored suggesting a strong probability of co-infection (kaya *et al.,* 2023). The CSU affects 0.5 to 1.5% of residents. However, 90% of chronic urticaria patients failed to be diagnosed. Around 20% of the universal inhabitants may develop urticaria at least one time throughout their lifetime. Although the diagnosis of urticaria is not difficult, yet, the discovery of its cause is not easy and demands careful inspection to find diagnostic signs and exclude critical medical conditions (Kolkhir *et al.,* 2016). The outcome of our study is to judge the correlation between the enteric parasites and *H.Pylori* antigen in patients diagnosed with CSU as a case group compared to non-diseased participants as the control group.

MATERIALS AND METHODS

Study Design:

A case-control study has been done at the Diagnostic and Research Unit of Parasitology (DRUP) at the Department of the Medical Parasitology, Faculty of Medicine, Cairo University, patients were selected from Dermatology Outpatient clinics from December 2023 to July 2024.

Study Population:

The population of the current study was distributed into two groups (40 participants each): the case group was selected from the Dermatology Outpatient clinics complaining of chronic spontaneous urticaria and the control group was without urticaria and was clinically free.Inclusion criteria involved both sexes, aged above 18 years complaining of chronic spontaneous urticaria that lasts more than 6 weeks, and patients who were not on antibiotics and/or anti-parasitic medications within the last month before the screening. Other causes like the use of medications, other infections, and psychological distress were rejected from the research.

History taking including symptoms and signs of urticaria, abdominal pain, diarrhea, fever, and headache in addition to clinical examination were taken from both case and control groups. Stool samples were collected and kept in an ideal-covered plastic cup. The stool samples were inspected macroscopically and then were

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divided into 2 parts: the first part was kept for microscope examination and the other part was stored at -20℃ for subtyping at the molecular level

Microscopic Examination and Staining:

The first part was examined microscopically using 3 different methods; first by 1- a wet mount which was done by placing stool on the slide and adding either a drop of saline or a drop of iodine, the second technique is 2- formalin-ethyl acetate sedimentation which was done to visualize different parasitic stages under a microscope and 3- modified Ziehl-Neelsen (MZN) stain which was done through the following steps: first a thin fecal smear was taken from the sediment produced by the sedimentation technique, which was left in the air to dry, then was fixed with absolute alcohol, and finally stained with the MZN stain for the detection of *Cryptosporidium* oocysts. Oval to spherical oocysts with diameters ranging from 4 to 5 µm, with pink to red color were detected against a blue background (El Naggar *et al.,* 2006).

Immunochromatographic Test (ICT):

An immunochromatographic test (ICT) for *H pylori* copro-antigen was done from the fresh samples using commercially available copro-immunoassays. The rapid diagnostic tests, ABON TM One Step *H. Pylori* Antigen TestDevice (Faeces) (Abon Biopharm, Hangzhou Co., Ltd., China) were used, following the manufacturer’s instructions (Agbor *et al.,* 2018)**.** It is a rapid one-step ICT: if the control and test lines were seen, the sample is considered positive, if only the control line was detected the sample is considered negative, and invalid if no lines were seen. Also, lines that appeared after 15 minutes or later had no diagnostic value and were not used for assessment.

PCR and Genotyping:

DNA was isolated from 200 mg of each stool sample positive for *Blastocystis* utilizing a DNA extraction kit (PSP Spin Stool Kit, Stractec Molecular, Berlin, Germany), tracking the manufacturer’s guidelines. Then extracted DNA was utilized to amplify Small subunit ribosomal RNA (SSU rRNA) genes with nested PCR. The product is approximately∼1100 base pairs in length. The first 1stprimer set comprised the forward primer forward A, GCTTA TCTGGTTGATCCTGCCAGTAGT, and reverse A, TGATCCTTCCGCA GGTTC ACCTA; (Yoshikawa *et al.,* 2000). The first PCR conditions were done as follows: initial denaturation was carried out at a temperature of 94 °C for 300 seconds, denaturation in which 30 cycles was carried out at a temperature of 94°C for 60 seconds, annealing was carried out at a temperature of 54 °C for 60 seconds, the extension was carried out at temperature 72 °C for 90 seconds, and last extension was carried out at temperature 72 °C for 600 seconds. While the primers forward B (5′-GGA GGT AGT GAC AAT AAA TC-3′) and reverse B (5′-ACT AGG AAT TCC TCG TTC ATG-3′) (Wong *et al.,* 2008) were used in the second PCR. The PCR circumstances were as follows: the initial denaturing stage was carried out at a temperature of 94 °C for 300 seconds, then denaturation in which 35 cycles were carried out at a temperature of 94°C for 60 seconds, annealing step was carried out at a temperature of 49°C for 60 seconds, extension stage was carried out at a temperature 72°C for 90 seconds, and last extension was carried out at a temperature 72°C for 600 seconds. Every tube contained 25 µL reaction which was composed as follows: 2 µL templates (extracted DNA represents the raw material in the primary reaction and PCR product from the 1st reaction represents the raw material in the secondary reaction). 1.5 mM MgCl2, 1x PCR buffer, 0.2 mM dNTPs, 2.5 U Taq DNA polymerase, and 1 M of each primer (Thermo Scientific™, Vilnius, Lithuania). The final PCR product is approximately (1100 bp) in length and the type of DNA ladder marker used was a 100-bp DNA ladder marker which was separated on 1.5 % agarose gel

where agarose concentration 1.5gram in each 100 ml buffer solution, stained with ethidium bromide and seen under a transilluminator which emits high levels of Ultra Violet radiation through viewing surface the representative *Blastocystis* RFLP banding patterns of subtype 3 were produced by RsaI endonuclease. The PCR products have entered the purification stage using a Geneclean II Kit (Bio 101). Then the DNA after purification was processed with RsaI in a 20 µl reaction ingredients composed as follows: 8 µl of DNA solution, 2 µl of 10 × buffer, 0.5 µl (5 U) of restriction endonuclease (Promega Corp.), and 0.2 µl of the bovine serum albumin solution (10 mg/ml) and 9.3 µl of distilled water was incubate at 37°C for 180 seconds. After that the unknown DNA produced by RsaI endonuclease was separated according to size using gel electrophoresis with a ladder its size was a 100-bp ladder (New England BioLabs, Inc.) in Tris-borate running buffer and 1.5% agarose gels. Ethidium bromide which is a fluorescent dye was used to visualize unknown DNA and the approximate sizes of the unknown DNA fragments were assessed.

To differentiate between the pathogenicity of *Entamoeba* species PCR was done for positive samples, where DNA was extracted from stool samples using by Favor Prep Stool DNA Isolation Mini Kit (Favorgen Biotech Corporation, Taiwan) according guidelines of the manufacturer. Then the small-subunit rRNA gene sequence in extracted DNA was ampilified using Multiplex PCR (mPCR) four primers were used in the multiple-PCR reaction: forward common *Entamoeba* primer, EntaF (5′ATGCACG AGAGCGAAAGCAT′ 3), with 3 *Entamoeba*-specific reverse primers, Ehr (5′GATCTAGAAACAATGCTTCTCT′3) for *Entamoeba histolytica*, EdR (5′CA CCACTTACTATCCCTACC′3) for *Entamoeba dispar*, and EmR (5′TGACC GGAGCCAGAGACAT′3) for *Entamoeba moshkovskii*. Optimum conditions occur in one reaction mixture. The common forward primer (EntaF) combined with each of the 3 reverse primers (EhR, EdR, and EmR) in a one-reaction mixture and under identical conditions. The reaction mixture included 10 µl of extracted DNA samples, 200 µM of each deoxynucleoside triphosphate, 0.1 µM of each forward and reverse primer, 0.5 U of Taq polymerase, 6 mM MgCl2, and 1× Taq buffer. Amplification of each specific DNA fragment to a particular species initiated with an initial denaturation at a temperature of 94°C for 180 seconds, then denaturation in which 30 cycles were carried out at a temperature of 94°C for 60 seconds, annealing was carried out at a temperature of 58°C for 60 seconds, then the extension was carried out at a temperature 72°C for 60 seconds, finally a last extension was carried out at temperature of 72°C for 420 seconds. After that, the products were seen with fluorescent dye ethidium bromide on 1.5% agarose gels using electrophoresis (Hamzah *et al*., 2006).

Statistical Analysis:

Data were coded and entered using the statistical package for the Social Sciences (SPSS) version 28 (IBM Corp., Armonk, NY, USA). Data was summarized using mean, standard deviation, median, minimum, and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. A Chi-square ($\chi 2$) test was used to compare categorical data. The accurate test was performed instead when the estimated frequency was less than 5 (Chan, 2003). *P*-values less than 0.05 were considered statistically significant.

Ethical considerations: Ethical approval was attained from the Faculty of Medicine, Cairo University.

RESULTS

Our study involved Eighty participants and they attended DRUP at the Parasitology Department, Faculty of Medicine, Cairo University. They were

classified into two groups (40 participants each): the case group with chronic spontaneous urticaria and the control group without urticaria.

The age and sex in both case and control groups were assessed statistically and showed no significant statistical association.

The distribution of positive *H. pylori* antigen in stool among the study population was 24 (60%) among cases and 14 (35%) among controls revealing a significant association (*P*=0.025). The presence of total enteric parasites in the stool within the study population was 17/40 (42.5%) within cases and 6 (15%) within controls revealing a significant association (*P*=0.07).

According to the enteric parasites found in the study; the overall detection rate of *Blastocystis* spp., *Cryptosporidium* spp., and *Entamaeba* spp. were detected in 14 (35%), 4 (10%), and, 2 (5%) respectively in the case group, The *Blastocystis* spp. positive patients were higher in cases compared to controls, with a distribution of 14 (35%) in cases and 6 (15%) in controls revealing a statistically significant difference (*P*=0.039). However, *Cryptosporidium* spp. and *Entamoeba* spp. among the research population showed no statistically significant difference. Data was recorded in Tables 1. In our study, one *Blastocystis* ST (ST3) was identified from stool samples by the nested PCR SSU rDNA sequences followed by REFLP (Fig. 1). Out of the 40 cases presented with chronic spontaneous urticaria two positive samples of *Entameba* were identified using multiplex PCR, and we found that one is *E. dispar* and the other one is *E. histolytica* (Fig. 2).

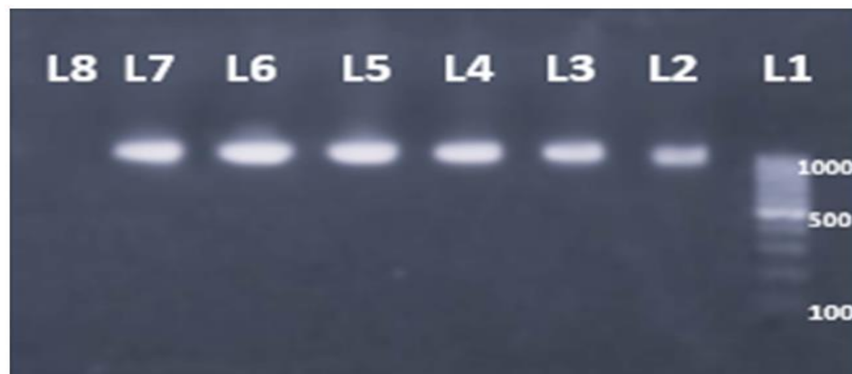

Fig. 1: Agarose gel electrophoresis for the products of the nested PCR targeting the SSU rDNA gene of Blastocystis species at 1100bp. Lane 1: 100 bp DNA molecular weight marker "ladder". Lanes 2-6: positive samples; Lane 7: Positive control; Lane 8: Negative control**.**

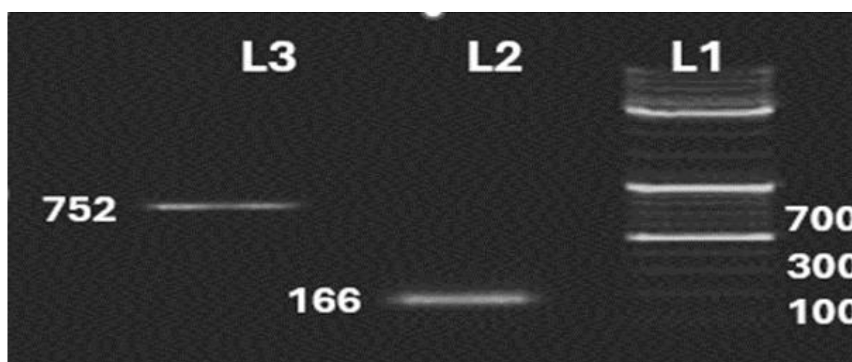

Fig. 2: Agarose gel electrophoresis revealed multiplex PCR ssu-rRNAgene products of Entamoeba. Lane 1 ladder marker: 100 bp. Lanes 2: positive *E histolytica* samples (166 bp). Lane 3 : positive *E. dispar* sample (752 bp).

However, the 4 *Cryptosporidium* oocyst samples in cases were detected by the MZN stain. At the time of the screening, 21(52.5%) cases out of 40 reported gastrointestinal symptoms, of which 10/40(25%) complained of upper abdominal pain, 11/40(27.5%) complained of diarrhea, however, some cases experienced other manifestations as; 10/40(25%) of the case group had a fever and 13/40(32.5%) had a headache. Data was recorded in Table 2.

Out Of 40 cases, in terms of *H.pylori* positivity, we found that there was an insignificant difference concerning enteric parasite species, although, among the *H.pylori* positive samples; 12/24(50%) were co-infected with intestinal parasites, and the concomitant infection was most frequent with *Blastocystis* spp. 9/24(37.5%), followed by *Cryptosporidium* spp. 3/24 (12.5%), and the least co-infection was with *Entamoeba dispar* 1/24 (4.2%). Data was recorded in Table 3.

In the cases group, we found that age, sex, and clinical manifestations were not statistically significant regarding the presence of *H. pylori* antigen or not. According to age, *H. pylori-positive* samples were higher in the age group 21-30 years (33.3%) while *H. pylori* negative samples were higher in patients aged more than 50 years (37.5 %). However, females 14/24 (58.3%) were more frequent than males 10/24 (41.7%) in positive *H. pylori* cases. Regarding the clinical manifestations, *H.pylori*-infected cases had a higher percentage of complaints compared to uninfected cases. Data was recorded in Table 4.

Table 1: Comparison between cases and control according to age, sex, *H.pylori* antigen, and intestinal parasitic infections positivity.

		Cases		**Control**		***P* value**
		Count	**%**	**Count**	**%**	
Age	≤ 20	4	10.0%	7	17.5%	0.574
	21–30	11	27.5%	9	22.5%	
	31–40	10	25.0%	12	30.0%	
	41–50	3	7.5%	5	12.5%	
	> 50	12	30.0%	7	17.5%	
Sex	Male	19	47.5%	16	40.0%	0.499
	Female	21	52.5%	24	60.0%	
***H. pylo*ri antigens**	Positive	24	60.0%	14	35.0%	0.025*
	Negative	16	40.0%	26	65.0%	
***Blastocystis* spp.**	Positive	14	35.0%	6	15.0%	0.039*
	Negative	26	65.0%	34	85.0%	
***Cryptosporidium* spp.**	Positive	4	10.0%	0	0.0%	0.116
	Negative	36	90.0%	40	100.0%	
Entamoeba dispar	Positive	1	2.5%	0	0.0%	1
	Negative	39	97.5%	40	100.0%	
Entamoeba histolytica	Positive	1	2.5%	0	0.0%	1
	Negative	39	97.5%	40	100.0%	
Total parasitic infections	Positive	17	42.5%	6	15.0%	0.007*
	Negative	23	57.5%	34	85.0%	

***: Significant (*P*<0.05)**

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Table 2: Distribution of Demographic, and clinical data, *H. pylori*, and intestinal parasites among patients with chronic spontaneous urticaria.

		Count	%
Age	20	4	10.0%
	21-30	11	27.5%
	31-40	10	25.0%
	41-50	3	7.5%
	> 50	12	30.0%
Sex	Female	21	52.5%
	Male	19	47.5%
***H pylori* infections**	Positive	24	60.0%
	Negative	16	40.0%
***Blastocystis* spp.**	Positive	14	35.0%
	Negative	26	65.0%
Entameba dispar	Positive	1	2.5%
	Negative	39	97.5%
Entameba histolytica	Positive	1	2.5%
	Negative	39	97.5%
***Cryptosporidium parvum* oocyst**	Positive	4	10.0%
	Negative	36	90.9%
fever	Positive	10	25.0%
	Negative	30	75.0%
headache	Positive	13	32.5%
	Negative	27	67.5%
Abdominal pain	Positive	10	25.0%
	Negative	30	75.0%
Diarrhea	Positive	11	27.5%
	Negative	29	72.5%

Table 3: The relationship between the positivity of *H. pylori* and intestinal parasites among case group.

		***H.pylori* antigens**				***P* value**
		Positive		**Negative**		
		Count	**%**	**Count**	**%**	
***Blastocystis* spp.**	Positive	9	37.5%	5	31.3%	0.685
	Negative	15	62.5%	11	68.8%	
Entamoeba dispar	Positive	1	4.2%	0	0.0%	1
	Negative	23	95.8%	16	100.0%	
Entamoeba histolytica	Positive	0	0.0%	1	6.3%	0.400
	Negative	24	100.0%	15	93.8%	
Cryptosporidium oocysts	Positive	3	12.5%	1	6.3%	0.638
	Negative	21	87.5%	15	93.8%	
	Negative	23	57.5%	34	85.0%	

Table 4: The relationship between the positivity of *H. pylori* and demographic and clinical data among case group.

		***H. pylori* antigens**				***P* value**
		Positive		**negative**		
		Count	**%**	**Count**	**%**	
Age	≤ 20	2	8.3%	2	12.5%	0.512
	21–30	8	33.3%	3	18.8%	
	31–40	5	20.8%	5	31.3%	
	41–50	3	12.5%	0	0.0%	
	> 50	6	25.0%	6	37.5%	
Sex	Male	10	41.7%	9	56.3%	0.366
	Female	14	58.3%	7	43.8%	
Fever	Positive	8	33.3%	2	12.5%	0.263
	Negative	16	66.7%	14	87.5%	
Headache	Positive	7	29.2%	6	37.5%	0.581
	Negative	17	70.8%	10	62.5%	
Abdominal pain	Positive	7	29.2%	3	18.8%	0.711
	Negative	17	70.8%	13	81.3%	
Diarrhea	Positive	7	29.2%	4	25.0%	1
	Negative	17	70.8%	12	75.0%	

DISCUSSION

Human co-morbidity by *H. pylori* and enteric parasites is a known major problem for patients, particularly in undeveloped nations. These two categories of pathogens mostly have similar influencing causes (Abd-Alghany *et al.,* 2022). Our study outcome is to detect intestinal parasites and *H. pylori* copro antigen among persons diagnosed with chronic spontaneous urticaria as a case group to find out the relation between them and chronic spontaneous urticaria development when compared to the control group.

We observed that the distribution of positive *H. pylori* in fecal samples was 24 (60%) in the cases and 14(35%) in the control revealing a significant association (*P*=0.025). Özdemir and Özgür. (2023) registered that the incidence of *H.Pylori* infection in patients suffering from chronic urticaria with no reason was 57.2%. In agreement with other studies that recorded that the incidence of *H.Pylori* infection in chronic spontaneous urticaria patients also ranged from 25% to 83% (Hook-Nikanne *et al*., 2000; Cuevas Acuna *et al*., 2006). Furthermore, Zuberbier *et al*. (2022) recommended that chronic spontaneous urticaria activity can be influenced by *H.pylori* infection. Another case-control study revealed that *H.Pylori* infection increased the risk of CSU by six times when compared to persons with negative *H.Pylori* antigen in the stool (Dennis *et al*., 2020). Buhner *et al*. (2004) explained that *H. pylori* infection can increase the gastric lining leaking, thus raising the contact with allergic substances within the gastrointestinal tract. Additionally, the immune system produces antibodies against the *H. pylori* infection which may promote histamine release within the skin. In our research, the frequency of enteric parasites in both cases and control was 17/40 (42.5%) and 6/40 (15%) respectively showing a significant association (*P*=0.07). In another study, parasitic infections were identified in 38.8% of chronic spontaneous urticaria patients and 11.1% of control persons showing a statistically significant difference (Dilek *et al*., 2012). Moreover, Zuberbier *et al*. (2014) observed that intestinal parasites were described as an underlying cause of CSU. In our study,

Blastocystis spp. was identified in 35% of cases and 15% of control recording a significant difference (*P*=0.039). Other parasites that demonstrated an association with the case group infection were *Cryptosporidium* oocyst and *Entamoeba* spp., showed no statistical significance. In 2023, Azami *et al*. found that cases of chronic spontaneous urticaria linked with *Cryptosporidium* spp. were described in a baby aged a year and 5 months in Isfahan, Iran. In another investigation, *Blastocystis* spp. was identified in 20% of individuals with CSU and 11.6% of the control showing a statistical significance. (Doğruman *et al*., 2009). According to Aykur *et al*. (2022), the *Blastocystis* rate was 31.9% in chronic spontaneous urticaria patients and 14.8% in control with a statistical difference (p<0.018). They explained that the antigens secreted by *Blastocystis* could stimulate skin urticaria owing to the increase in intestinal permeability (Dagci *et al*., 2002). *Blastocystis* antigens might provoke the stimulation of Th2 lymphocytes with the manufacture of certain cytokines such as interleukin causing an allergic response due to the production of immunoglobulin E (Lepczynska *et al*., 2016). In 2020, Bahrami *et al*. found that there was a connection between blastocystosis and the development of chronic spontaneous urticaria. Our analysis identified one *Blastocystis* subtype (ST3). Similarly, a patient infected with *Blastocystis* of subtype 3 presented with urticaria rash which was mentioned in a case report (Katsarou-Katsari *et al*., 2008).

In our study, 21 cases out of 40 (52.5%) reported gastrointestinal symptoms, of which 10/40 (25%) complained of upper abdominal pain, 11/40 (27.5%) complained of diarrhea, however, some cases experienced other manifestations; 10/40 (25%) of the cases had a fever and 13/40 (32.5%) had a headache. Similarly, another study recorded that the most important symptoms linked to *Blastocystis* infection in patients with urticaria involved diarrhea (24.1%), and abdominal colic (14.8%) (Hameed *et al.* 2011). According to Elhendawy *et al.,* (2021), stated that positive and negative *H pylori* chronic spontaneous urticaria patients exhibit similar clinical manifestations regardless of age and sex as observed in our study. In American research including 155 patients with CSU, 26.2% of the study patients had gastrointestinal complaints (Doong *et al*., 2017). Different explanations have been considered in the research to clarify the related gastrointestinal complaints in chronic urticaria patients. The rise in eosinophil and IgE levels suggests progressive activation of Th2 immune cells in the research population (Aitella *et al*., 2018).

In our research, we noticed that a correlation between *H. pylori-positive* samples and intestinal parasites showed no statistical significance. However, among the *H. pylori-positive* samples; 50% were co-infected with enteric parasites, and the concomitant infection was most frequent with *Blastocystis* spp. (37.5%), followed by *Cryptosporidium* spp. (12.5%), and the least co-infection was with *Entamoeba dispar* (4.2%). This agrees with an Egyptian study by Ahmed *et al.*, (2018), who stated more parasitic infections in *H. pylori-positive* patients. In addition, Yakoob *et al*., (2018), informed that patients infected with *H.pylori* had a higher likelihood to have intestinal parasites such as; *Blastocystis* spp. and *E.histolytica.* Also, Pomari *et al.,* (2020), discovered patients with enteric parasites had a higher risk of *H. pylori* infection than healthy persons, and that the most common co-infection was *H. pylori and Blastocystis*. Also, Gallab and Morsy. (2020) found *Blastocystis* spp. to be the most prevalent co-infection with *H. pylori* followed by *Cryptosporidium parvum*, followed by *Giardia lamblia*, and lastly *Entamoeba histolytica/dispar.* Additionally, Moro *et al.,* (2022), detected *H. pylori-blastocystis* co-infection in

40.9% of total patients. On the contrary, Sabah *et al.,* (2015), recorded that co-infection was more between *H. pylori* and *Entamoeba histolytica* and *Giardia lamblia*. Similarly, another study found that 52.5% co-infection between *H.pylori* and giardiasis (El-Badry *et al.,* 2017). Also, Ahmed *et al.,* (2018), detected 55.5% co-infection with *H. pylori* and *Entamoeba* spp. Additionally, Seid *et al*., (2018), recorded no substantial link between *H. pylori* and *E. histolytica*, although *Giardia lamblia* had a statistical significance. Taghipour *et al.,* (2022), recorded a greater percentage of *H. pylori* and *Giardia* co-infection showing no significant difference. However, Rahi and Fadhil. (2021), stated that *H. pylori and Cryptosporidium parvum* co-infection was 32%. Habeeb and Abed, (2021), found that the co-infection rate between *H. pylori* and *Entamoeba histolytica* (29.03%) was greater than that of *H. pylori* and *Giardia lamblia* (26.66%). However, Kaya *et al.,* (2023), found a statistical significance in co-infection between *Entamoeba histolytica and H. Pylori.* Yet, Bin-Hameed and Barajash, (2023) found that the most prevalent co-infections with *H.pylori* were *Giardia* and *Entamoeba histolytica* with insignificant differences. While, Hassan *et al.,* (2024), detected *H. pylori-Entamoeba histolytica* co-infection to be the most common by 93.3%. Additionally, Mina *et al.,* (2024), stated that the percentage of co-infection with parasitic infections and *H. pylori* was 11.8% and *H. pylori* co-infected with *Entamoeba histolytica* (77.1%) was the most frequent parasite

The current study noticed that age, sex, and clinical manifestations were not statistically significant in patients with *H.pylori* infection. According to age, *H. pylori-positive* samples were higher in the age group 21-30 (33.3%). In contrast, Bordin *et al.,* (2022), stated that the *H. pylori*-infected personnel were aged 40 and more. Kaya *et al.,* (2023), also noticed the highest infection with *H. pylori* frequency was in the 41-50 age range. In addition, Chen *et al.*, (2023), it was documented that *H. pylori* prevalence increases between the ages of 45 and 64. Concerning sex distribution, females had a greater percentage of positive *H.pylori* samples (58.3%) vs (41.7%) of males . Similarly, Seo *et al.,* (2020), recorded that the frequency rate of *H. pylori* infection was 68.5% in females versus 59.3% in males. Also, Cui *et al.,* (2021), stated that females were more likely to be infected with *H.pylori* than males. In addition, Kaya *et al.,* (2023), recorded that 76.6% of the participants having *H. pylori* infection were females. Also, Almashhadany *et al.,* (2023), mentioned that *H. pylori-positive* samples were higher in females 21.9% than in males 17.2%. In contrast, Corojan *et al.,* (2020) noticed that *H. pylori* prevalence was 40.53% in females & 41.35% in males with no differentiation regarding sex. Mezmale *et al.,* (2021), stated that males (66.3%) had a higher *H. pylori* infection rate than females (57.4%). Males had a higher rate of *H. pylori-positive* samples than females, which was also recorded by Wu *et al*., 2022. Regarding the clinical manifestations, the proportion of complaints such as fever, headache, abdominal pain, and diarrhea was more prevalent in patients infected with *H. pylori* than those who were not infected with *H.pylori.* Abd El Hameed *et al.,* (2021), reported in their study that among 150 patients of both sexes complaining of gastrointestinal symptoms, the most common clinical presentation in the group infected with *H. pylori* was abdominal pain (85.3%), however, diarrhea wasn’t a prominent symptom. In contrast, Morsy *et al.,* (2024), recorded a substantial link between diarrhea and *H.pylori* (P <0.001).

CONCLUSION:

H.Pylori and enteric parasites are detected at a greater rate in patients suffering from chronic spontaneous urticaria than in healthy control. This observation prompts further investigation

to find a possible connection between the CSU and infection with *H. Pylori* and enteric parasites. Thus, frequent stool tests for antigens of *H. pylori* and enteric parasites are necessary for chronic spontaneous urticaria patients particularly in individuals resistant to standard therapy of chronic urticaria or in individuals with associated gastrointestinal symptoms.

Declarations:

Ethical Consideration: Ethical approval was attained from the Faculty of Medicine, Cairo University; Ethical Approval Committee Number A-35-2024.

Competing interests: The authors declare no competing interests

Author's Contributions: all authors participated equally in practical work and writing manuscript of this study.

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