The impact of sex, age, and season on the prevalence of *Helicobacter pylori* infection in Lithuania

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² Public institution Kaunas City Outpatient Clinic, Pramonės pr. 31, Kaunas 51270, Lithuania The aim of this study was to identify the prevalence of *H. pylori* infection in adult patients in Kaunas, Lithuania, and to assess the dependence of *H. pylori* infection on sex, age, and seasonality. A total of 5799 patients were tested for the presence of *H. pylori* by ELISA. Three different tests were used: IgA and IgG antibodies against *H. pylori* from blood serum and antigens of *H. pylori* from faeces. The results revealed that the frequency of *H. pylori* infection varied depending on the test (31.8% for IgG, 16.7% for IgA, and 22.9% for antigens test). No significant association between sex, season, and the prevalence of *H. pylori* was observed. However, our findings indicate a significant increase in the prevalence of *H. pylori* infection with age. This study is the first report about the impact of sex, age, and season on the prevalence of *H. pylori* infection in Lithuania.

Keywords: *Helicobacter pylori*, antibody, antigen, sex, age, season

INTRODUCTION

Helicobacter pylori is a Gram-negative helical bacterium that colonises the stomach. This bacterium can lead to various gastrointestinal disorders and is considered a causative agent of gastric cancer (Mladenova, 2021; Almashhadany et al., 2023). The rate of *H. pylori* infection is more than 50% of human populations worldwide (Park et al., 2021). However, many *H. pylori* infections are asymptomatic (Almashhadany et al., 2023). The transmission of *H. pylori* is still not fully understood. New infections of *H. pylori* can pass from human to human directly or through environmental con-

tamination (Kayali et al., 2018). Furthermore, poor hygienic conditions may also contribute to the spread of *H. pylori* (Breckan et al., 2016).

The recent pandemic improved hygiene habits of the population thus helping to prevent the spread of many infections (Macaraan, 2022). However, the resulting situation has left residual effects: it slowed down the pace of life and physical activity; conditions were created for employees to work from home and for students to study remotely; it introduced the possibility for the representatives of health care sector to provide consultations remotely avoiding contact examination of patients. After three years of neglecting health ailments, people are finally paying attention to the symptoms they previously ignored. The visits of patients to the gastroenterologist reveal that more and more

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patients are diagnosed with *H. pylori* infection, which is manifested by nausea, rising acids, and acute stomach pains (Almashhadany et al., 2023; Lupu et al., 2023). Timely diagnosis of the disease and prompt treatment, which would prevent the complications of the infection and the subsequent consequences of the disease, are critical (Mărginean et al., 2022).

The infection of *H. pylori* can be diagnosed by non-invasive tests such as the urea breath test, stool antigens, the serological test, and molecular tests (Mărginean et al., 2022). According to the recommendations of the World Health Organization, it is important not only to properly treat *H. pylori* infection but also to diagnose the existing disease accurately and timely. The aim of this study was to identify the prevalence of *H. pylori* infection in adult patients in Kaunas, Lithuania. A further objective of this work was to assess the dependence of *H. pylori* infection on sex, age group, and season.

MATERIAL AND METHODS

This study was performed on 5799 patients visiting the Kaunas City Outpatient Clinic (Lithuania) within a period of one year (1 December 2021-31 November 2022). A semi-quantitative in vitro enzyme-linked immunosorbent assay (ELISA) was chosen to detect IgA and IgG antibodies against H. pylori from blood serum and antigens of H. pylori from the faeces. All samples were tested with a fully automated ELISA Analyzer I-2P (EUROIMMUN, Germany). Serology (IgG and IgA) was performed with the Anti-Helicobacter pylori ELISA kit (EUROIMMUN, Germany) according to the manufacturer's instructions. Antigens of H. pylori in human faeces have been detected using CoproELISATM H. pylori test kit (Savyon Diagnostics Ltd., Israel) as per the manufacturer's instructions. The patients were included in the process only once. The patients' personal data were not used to ensure anonymity.

The research was based on the analysis of the data from the clinic's registers. The data were analysed using MS Excel software. All patients enrolled in this study were grouped based on sex (female and male) and age $(1. \le 19 \text{ years}; 2. 20-39 \text{ years}; 3. 40-59 \text{ years}; 4. \ge 60 \text{ years})$. The categorical variables were analysed with a chi-squared test (Statistica for Windows, v. 7.0, Statsoft, Tulsa, OK, USA). The observed differences were considered to be significant when p < 0.05.

RESULTS AND DISCUSSION

In this study, we report dependence of *H. pylori* infection on sex, age group, and season in adult patients in Lithuania. Several previous studies focused on antibiotic resistance of *H. pylori*, the distribution of different *H. pylori* genotypes in Lithuania, management *H. pylori*-related disease, and on the relation of *H. pylori* infection with comorbidities and other symptoms (Chalkauskas et al., 1998; Miciuleviciene et al., 2008; Kupcinskas, Leja, 2014; Jonaitis et al., 2016; Dargiene et al., 2018; Jonaityte et al., 2021).

This study evaluated data from 5799 patients, 4191 of whom were tested for IgG, 725 for IgA, and 883 for antigens. The overall prevalence of *H. pylori* infection was 28.6% (1657/5799). We found found that 31.8% (1334/4191) were positive for H. pylori IgG antibodies, 16.7% (121/725) were positive for H. pylori IgA antibodies, and 22.9% (202/883) were positive for H. pylori antigens. The percentage of H. pylori infection varies between 20% and 80% worldwide (Hooi et al., 2017). The most useful test in clinical practice is the detection of H. pylori antigen in faeces (Mărginean et al., 2022), which allows the diagnosis of active H. pylori infection (Best et al., 2018). Meanwhile, the positive result of the serological test does not necessarily indicate acute infection and only the IgG test can indicate chronic infection (Mărginean et al., 2022).

The prevalence of *H. pylori* infection varies across different populations and is associated with several risk factors, including sex (de Martel, Parsonnet, 2006). Previous studies found that there are sex differences in *H. pylori* infection rates, some suggesting that females may be more susceptible to the infection, while

others finding no significant difference between males and females (Zamani et al., 2018; Khoder et al., 2019; Almashhadany et al., 2023). When assessing the distribution of *H. pylori* infection between males and females in Kaunas (Lithuania), the infection rate for females was found to be 27.6%, whereas the infection rate for males was 30.5% ($\chi^2 = 5.226$, p < 0.05) (Table 1). However, when evaluating the results of different tests (IgG, IgA, and Ag), a chi-square test of independence showed that there was no significant association between sex and the prevalence of *H. pylori* (p > 0.05). Within the population investigated, females accounted for approximately two-thirds (67.6%), while males accounted for one-third (32.4%). This indicates that women tend to take much more care of their daily well-being and health.

Age is specified as a risk factor for *H. pylori* infection (Öztekin et al., 2021). The prev-

alence of *H. pylori* infection among children increases with age within the first ten years of life (Mhaskar et al., 2013). In adults, age also affects the incidence of *H. pylori* infection (Saadi, Saeed, 2018; Zamani et al., 2018; Almashhadany et al., 2023). Our findings indicate a significant increase of *H. pylori* infection with age (Table 2). The highest prevalence rate (33.7%) was observed among participants aged between 40 and 59 years. Previous studies conducted in other countries revealed similar results (Breckan et al., 2016; Saadi, Saeed, 2018; Khoder et al., 2019; Haq et al., 2020).

In the current study, the data were analysed to evaluate the dependence of the frequency of H. pylori infection on different seasons (Table 3). The highest frequency of the infection was determined with the IgG test in spring (32.8%, p > 0.05) and with IgA and Ag tests in summer (22.3%, p > 0.05, and 29.4%,

Table 1. Prevalence of H.	pylori infection and its correlation v	vith sex

	Number of positive/number of tested			.2 -n d 6 lu
	Female	Male	Total	χ^2 and p values
IgG	873/2815	461/1376	1334/4191	$\chi^2 = 2.642$,
	(31.0%)	(33.5%)	(31.8%)	p = 0.104
IgA	78/484	43/241	121/725	$\chi^2 = 2.345$,
	(16.1%)	(17.8%)	(16.7%)	p = 0.557
Ag	132/620	70/263	202/883	$\chi^2 = 2.969$,
	(21.3%)	(26.6%)	(22.9%)	p = 0.085
Total	1083/3919	574/1880	1657/5799	$\chi^2 = 5.226$,
	(27.6%)	(30.5%)	(28.6%)	p = 0.022

Table 2. Prevalence of H. pylori infection and correlation with age groups

	Number of positive/Number of tested			w² and a values		
	≤19 years	20-39 years	40-59 years	≥60 years	χ^2 and p values	
IgG	17/105	360/1141	499/1295	458/1650	$\chi^2 = 51.3017$,	
	16.2%	31.6%	38.5%	27.8%	<i>p</i> < 0.00001*	
IgA	1/42	45/229	47/241	28/213	$\chi^2 = 10.9237$,	
	2.4%	19.7%	19.5%	13.1%	$p = 0.01215^*$	
Ag	5/17	60/234	76/312	61/320	$\chi^2 = 4.4522$,	
	19.4%	25.6%	24.4%	19.1%	p = 0.21660	
Total	23/164	465/1604	622/1848	547/2183	$\chi^2 = 53.7790$,	
	14.0%	29.0%	33.7%	25.1%	$p < 0.00001^*$	

^{*} Indicates the result which is significant at p < 0.05.

	Number of positive/number of tested			w ² and a values		
	winter	spring	summer	autumn	χ^2 and p values	
IgG	327/1011	365/1112	278/885	364/1183	$\chi^2 = 1.3139$,	
	32.3%	32.8%	31.4%	30.8%	p = 0.7258	
IgA	22/183	28/168	35/157	36/217	$\chi^2 = 6.4145$,	
	12.0%	16.7%	22.3%	16.6%	p = 0.0931	
Ag	52/208	53/212	57/194	40/269	$\chi^2 = 15.5002$,	
	25.0%	25.0%	29.4%	14.9%	$p = 0.0014^*$	
Total	401/1402	446/1492	370/1236	440/1669	$\chi^2 = 6.3915$,	
	28.6%	29.9%	30.0%	26.4%	<i>p</i> < 0.0940000	

Table 3. Prevalence of H. pylori infection and its correlation with the season

p < 0.05 respectively). Monthly prevalence of H. pylori depending on sex and different age groups are graphically depicted in Figs 1 and 2. A chi-square test of independence was performed to examine the relation between sex and prevalence of H. pylori infection in different months. The correlation between these variables was not significant (p > 0.05). However, there was a significant correlation be-

tween age and month-dependent prevalence of H. pylori (p < 0.05). with the correlation between the season and H. pylori infection has been studied by other researchers (Raschka et al., 1999; Haq et al., 2020; Hemati et al., 2021; Almashhadany et al., 2023). The authors also noted no significant correlation between the season and the incidence of H. pylori infection.

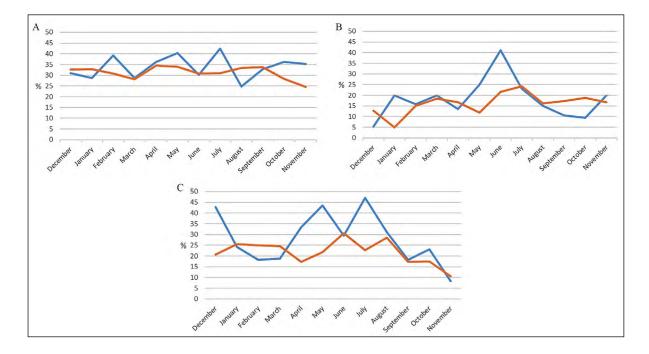


Fig. 1. Month-dependent prevalence of *H. pylori* depending on sex. Blue lines represent males, orange lines females. A – IgG test, B – IgA test, C – antigen test

^{*} Indicates the result which is significant at p < 0.05.

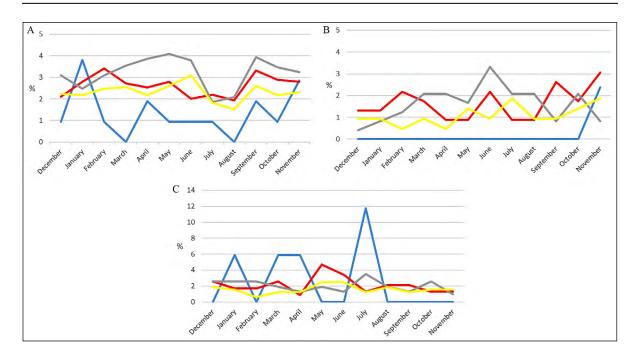


Fig. 2. Month-dependent prevalence of *H. pylori* depending on age. Blue lines represent participants under 19 years of age, red lines – between 20 and 39 years, grey lines – between 40 and 59 years, and yellow lines participants aged 60 and above. A – IgG test, B – IgA test, C – antigen test

CONCLUSIONS

To the best of the authors' knowledge, this study is the first report about the impact of sex, age, and season on the prevalence of *H. pylori* infection in Lithuania. Our results indicate that sex and season do not have a significant correlation with *H. pylori* infection, but we identified a significant impact of age on the prevalence of *H. pylori* infection. Further research is needed to better understand how different factors affect the rates of *H. pylori* infection.

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LYTIES, AMŽIAUS IR SEZONO ĮTAKA HELI-COBACTER PYLORI INFEKCIJOS PAPLITI-MUI LIETUVOJE

Santrauka

Šio tyrimo tikslas buvo nustatyti *H. pylori* infekcijos paplitimą tarp suaugusių asmenų Kaune ir įvertinti infekcijos priklausomybę nuo lyties, skirtingo amžiaus ir sezoniškumo. Iš viso 5 799 pacientai buvo ištirti dėl H. pylori infekcijos ELISA metodu. Tam buvo atlikti trys skirtingi tyrimai – H. pylori IgA ir IgG antikūnų iš kraujo serumo ir H. pylori antigenų iš išmatų testai. Rezultatai atskleidė, kad nustatytos H. pylori infekcijos dažnis skyrėsi priklausomai nuo atliekamo testo (31,8 % IgG, 16,7 % IgA ir 22,9 % antigenų tyrimas). Reikšmingo ryšio tarp lyties ar sezono ir H. pylori paplitimo nepastebėta. Tačiau gauti rezultatai rodo, kad su amžiumi H. pylori infekcijos paplitimas gerokai išauga. Tai pirmas tyrimas apie lyties, amžiaus ir sezono įtaką H. pylori infekcijos paplitimui Lietuvoje.

Raktažodžiai: *Helicobacter pylori*, antikūnas, antigenas, lytis, amžius, sezonas