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Detection of *Helicobacter pylori* using Giemsa staining in chronic tonsillitis patients: cross-sectional study

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Abstract

Background *Helicobacter pylori* (*H. pylori*) is a well-known bacteria that is recognized as the most prevalent infection in humans and is present in the majority of people in the world.

Aim To investigate the presence of an extragastric reservoir of *Helicobacter pylori* in tonsillectomy specimens to ascertain whether it is an etiologic factor in the development of chronic tonsillitis.

Subjects and methods The study included 100 patients of both sexes with chronic tonsillitis who visited the ENT (ear, nose, and throat) outpatient department and tested positive for *H. pylori* antigen in stool and tonsillectomy materials. Giemsa stain was used. The population was patients with chronic tonsillitis who had been diagnosed based on physical examination and history-taking at the (Oto-Rhino-Laryngology) outpatient clinic.

Results It is shown that patients with positive *H. pylori* had considerably more acute tonsillitis attacks per year than those with negative *H. pylori*. Examining the patients with Giemsa modification staining revealed 40% of them to be positive.

Conclusion *H. pylori* was found in the tonsil tissue of 40% of the patients with chronic tonsillitis using the Giemsa modification staining method, suggesting that *H. pylori* may be contributing etiologic factors in the development of chronic tonsillitis.

Keywords *Helicobacter pylori*, Giemsa stain, Tonsillitis, Tonsillectomy

Background

It has been determined that more than 50% of people worldwide have a common infection brought on by the well-known pathogen *Helicobacter pylori* (*H. pylori*) [1].

H. pylori is a substantial contributor to the etiology of gastritis, gastric, and duodenal ulcers [2].

In agreement with the same mechanism of transmission, studies on *H. pylori* colonization in the oral mucosa and upper respiratory tract have been described [3].

In recent years, adenotonsillar tissue has started to be recognized as one of the reservoirs for microorganisms [4].

The stomach mucosa is where the gram-negative, spiral-shaped *H. pylori* bacteria are most frequently detected. Urea is broken down by the urease enzyme, which *H. pylori* produces into carbon dioxide and ammonia. Ammonium shields the stomach's acidic environment [5].

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If the infection, which usually affects infants, is left untreated, the host will die permanently.

Given that tonsillar tissue is a part of the lymphoid system and resembles the mucosa-associated lymphoid tissue of the stomach, an increasing number of researchers have recently looked into the prevalence and contribution of the *H. pylori* bacterium to the emergence of tonsil illnesses [6].

It has been proposed that the presence of *H. pylori* in tonsillar tissue may cause the tonsils to become inflamed [7].

Research suggests chronic tonsillitis causes the stomach to become infected again [8].

Pharyngeal tonsils become inflamed when someone has tonsillitis.

Acute tonsillitis is an infection of the tonsils brought on by a variety of bacteria or viruses, and it may also result in peritonsillar abscesses. Tonsil infections, such as chronic tonsillitis, are persistent and cause tonsil stones. When a person contracts tonsillitis on a regular basis, it is referred to as recurrent tonsillitis. Both chronic and recurrent tonsillitis, which can have a negative impact on a patient's quality of life, are characterized by recurrent tonsillitis [9].

Chronic tonsillitis, which affects both adults and children, is a major medical issue [10] and while the definition of severe recurrent tonsillitis varies, severity is described as five or more episodes of tonsillitis a year, symptoms for at least a year [11].

In order to determine if *Helicobacter pylori* is an etiologic factor in the development of chronic tonsillitis, we looked at its existence as an extra gastric reservoir in tonsillectomy specimens.

Methods

Study group

This study was carried out in patients attending the ENT (ear, nose, and throat) Department Outpatient clinic, from March 2021 until April 2022, the study included 100 patients of both sexes suffering from chronic tonsillitis and who were positive for *H. pylori*.

The Department of Oto-Rhino-Laryngology collected samples from patients with chronic tonsillitis who underwent tonsillectomy, were tested, and were willing to take part in the study by signing informed consent forms. The invasion of the bacterium was evaluated using the Giemsa stain. Chronic tonsillitis patients made up the population. With their ages ranging from between 5 and 25 years with a mean of 12.68 ± 7.19 years. There is a predominance of male patients in this study of which 74 were males (74%) and 26 were females (26%). All patients were exposed to complete history taking and ENT examination.

Methodology

The protocol of evaluation in this study was as follows:

Inclusion criteria

Patients of age (5–25 years) and both sexes, patients with features of chronic tonsillitis who will do a tonsillectomy operation, and patients with a positive history of *Helicobacter pylori* antigen in stool.

Exclusion criteria

Patients above 25 years and below 5 years of age, patients with acute tonsillitis, patients with cleft lips and/or cleft palate, immune-compromised patients (diabetic, chemotherapy and radiotherapy, renal, and hepatic failure), patients with congenital anemia, malabsorption or other gastrointestinal diseases, and patient refusal.

Methods

In this prospective investigation, 100 samples of tonsils from individuals with chronic tonsillitis (who were positive in stool for *Helicobacter pylori*) were collected. The inclusion criteria for elective tonsillectomy due to persistent tonsillitis were met by all recruited individuals. The tonsillectomy procedure was performed in the operating room. The pathology department examined the tonsillar tissue using Giemsa staining to determine whether *Helicobacter pylori* was present.

Tissue samples need to be transferred to the lab as soon as possible in a unique transport medium. For growth, *Helicobacter pylori* needs a microaerophilic atmosphere, enriched media, and an incubation period of up to 7 days. Other oral species frequently overgrow in these circumstances. Tonsillar tissue samples were marked and preserved in 10% formalin for 24 h in the pathology department. These samples were divided into 3-mm-thick pieces. These fragments were sent into an automated processing device, where paraffin-embedded tissue was created. The block has been ready. Tonsillar tissue samples were cut into 5- μ m slices, which were then hydrated with distilled water then incubated for 15 min in the stain, followed by thorough washing in water, dehydration, clarifying, and mounting. Giemsa 1% was then put on this slide for 10 min. After being meticulously cleaned, the slides were dried off and placed back on a rack. The staining test was carried out by the pathology laboratory of the Faculty of Medicine at Al-Azhar University, and the specialists there evaluated the samples. High power magnification was used to individually assess each slide for the presence or absence of *Helicobacter*-like organisms.

Examination

General examination and E.N.T examination including tonsil examination to show the signs of chronic tonsillitis including (congested anterior pillars, asymmetrical enlargement on both sides of tonsils, irregular shape, oozing pus when squeezing, and enlarged juglo-digastric lymph nodes).

Investigations

Routine investigations before tonsillectomy operation (complete blood count-Anti-streptolysin O titer-coagulation profile-ESR-CRP-H. pylori antigen in stool).

Data management and statistical analysis

Quantitative data were statistically represented in terms of minimum, maximum, mean, standard division (SD), and median. Comparison between different groups in the present study was done using Mann–Whitney test for comparing two nonparametric groups. Qualitative data were statistically represented in terms of number and percent. Correlation between various variables was done using the Spearman correlation coefficient (R). Reliability was calculated using Cronbach’s alpha and test–retest reliability correlation. A probability value (p value) less than or equal to (0.05) was considered significant. All statistical calculations were done using the computer program SPSS (Statistical Package for Social Science) statistical program version (21.0). Graphs were done using the SPSS statistical program version (21.0) and Microsoft Excel program version 2016.

Results

The present study was carried out on 100 patients of both sexes attending the ENT outpatient department suffering from chronic tonsillitis. The group comprised patients with chronic tonsillitis identified between March 2021 and April 2022 at the Oto-Rhino-Laryngology) outpatient clinic.

Samples were obtained using sequential sampling from all chronic tonsillitis patients who underwent a tonsillectomy at the Department of Oto-Rhino-Laryngology and signed informed consent forms.

From the measured variables the results of the study showed:

1. H. pylori prevalence among the studied patients showed that out of 100 chronic tonsillitis patients with positive H. pylori in stool, 40 patients were positive H. pylori in tonsillar tissue by Giemsa staining as shown in (Table 1).
2. The study included 100 patients between 5 and 25 years. Male patients are predominant in this study of which 77 were males (77%) and 23 were females (23%) attending the ENT outpatient department suffering from chronic tonsillitis who were positive for H. pylori antigen in stool and were examined in tonsillectomy specimens. The demographic distribution shows most of the patients were less than 10 years with a mean of 12 ± 6.77 years with 52% less than 10 years, 30% (10–20 years), and 18% more than 20 years as shown in (Table 2).
3. The most common clinical presentation of the studied patients was a sore throat in 95% of patients followed by a bad odor of breath in 58% of patients, lump sensation in 38%, hoarseness of voice in 37%, excess throat mucous in 36%, and heartburn in 18% of patients (Table 3).
4. There was statistical significance in the number of acute attacks of tonsillitis per year which was significantly more frequent in patients with positive H.

Table 1 H. pylori prevalence among the studied patients

H. pylori prevalence among the studied patients	Cases (n = 100)
Positive	40 (40%)
Negative	60 (60%)

This table shows H. pylori prevalence among the studied patients showed that out of 100 chronic tonsillitis patients with positive H. pylori in stool, 40 patients were positive H. pylori in tonsillar tissue by Giemsa staining

Table 2 Demographic distribution and clinical characteristics of the patients

Variable	Cases (n = 100)
Age (years) Mean \pm SD	12 ± 6.77
Less than 10 years	52 (52%)
10–20 years	30 (30%)
> 20 years	18 (18%)
Sex	
Female	23 (23%)
Male	77 (77%)

The study included 100 patients between 5 and 25 years. Male patients are predominant in this study of which 77 were males (77%) and 23 were females (23%) patients were less than 10 years old with a mean of 12 ± 6.77 years with 52% less than 10 years, 30% (10–20 years) and 18% more than 20 years

Table 3 Clinical presentation of the studied patients

Clinical presentation of the studied patients	Cases (n = 100)
Sore throat	95 (95%)
Hoarseness of voice	37 (37%)
Lump sensation	38 (38%)
Bad odor breath	58 (58%)
Heartburn	18 (18%)
Excess throat mucous	36 (36%)

pylori > 6 times per year in 19 patients out of 40 positive *H. pylori* patients (47.5%) compared to negative ones (Table 4).

- The Anatomic Pathology Department did the staining examination and the specimens were examined by the experts in the pathology Laboratory of the Faculty of Medicine, Al Azhar University. Each slide was separately evaluated for the presence or absence of *Helicobacter*-like organisms at high power magnification.
- H. pylori* colonization in the tonsillar tissue is shown in the studied cases by Giemsa stain (arrow) (Fig. 1).

Discussion

Numerous investigations have suggested that *H. pylori* may be able to colonize the tonsil.

According to Ozgun et al. [12], 48 (48%) of the 100 patients who underwent tonsillectomy tested positive for *H. pylori*, while 52 (52%) tested negative. According to Lin et al. [13] research, 33 (35%) of 94 patients treated for tonsillitis tested positive for *H. pylori*, whereas 61 (65%) tested negative. According to Wibawa et al. [14] 15.7% of 19 people with chronic tonsillitis had *H. pylori* positive test results. As a result, more researchers are now looking into the prevalence and significance of *H. pylori* in the etiology of chronic tonsillitis.

Table 4 History of frequency of acute attacks of tonsillitis per year

Acute attack frequency of tonsillitis per year	Positive <i>H. pylori</i> (n = 40)	Negative <i>H. pylori</i> (n = 60)	P
1–2 times	1 (2.5%)	28 (46.7%)	.001
3–4 times	8 (20%)	17 (28.3%)	
4–6 times	12 (30%)	9 (15%)	
> 6 times	19 (47.5%)	6 (10%)	

This table shows that the number of acute attacks of tonsillitis per year was significantly more frequent in patients with positive *H. pylori* compared to negative ones

An etiopathogenetic component in chronic tonsillitis may be the presence of *H. pylori* in tonsillar tissue, causing inflammation in the tonsils and re-infection of the gastric mucosa [15]. In the present investigation, tonsillectomy specimens from 100 instances of chronic tonsillitis with positive *Helicobacter pylori* antigen in stool were analyzed. Giemsa stain was used to assess the microorganism's invasion. Patients of both sexes with chronic tonsillitis were seen in the ENT outpatient department for clinical research.

Patients with chronic tonsillitis who had a history-taking and physical examination as part of an outpatient ORL (Oto-Rhino-Laryngology) appointment. The duration of the study was from March 2021 to April 2022. The patients' age range was between 5 and 25 years with a mean of 12.68 ± 7.19 years. There is a predominance of male patients in this study of which 74 were males (74%) and 26 were females (26%).

Our results may agree with the study by Asyari et al. [16] as they discovered that children aged 5 to 14 were the most frequently affected by chronic tonsillitis (44%). Also, in this study, the most predominant age group was from 5 to 15 years (71%). This study showed that 40% of the patients were positive for *H. pylori* by using Giemsa staining of the tested tonsillar tissues which agrees with the study by Ahmed Abdel Aziz [17] which had been shown that 56% of the studied patients were positive for rapid urease test and had *H. pylori* antigen in the stool.

This study agreed with the study by Ahmed Abdel Aziz [17]. The patient's age range was between 3 and 15 years with a mean of 5.6 ± 2.1 . Twenty-three of the studied patients were male (46%) and 27 were female (54%). The present study showed that the most common clinical presentation was sore throat (95%) followed by bad odor breath (58%). In accordance with our results, in the study by Asyari et al. [16], the most prevalent complaint was recurring swallowing pain, which was reported by 12 patients (48%), 7 times annually. All patients with chronic tonsillitis experienced lumps. Twenty-four patients (96%)



Fig. 1 The photograph showing the *H. pylori* colonization in the tonsillar tissue in the studied cases by Giemsa stain (arrow)

expressed concern about having poor breath (halitosis). This study does not agree with the study by Pourmoussa et al. [18]. Results revealed that among 104 (91.2%) youngsters, adenoid hypertrophy was the most prevalent manifestation. The symptoms of recurrent tonsillitis were further examined in another study by Singh et al. [19], and it was shown that only 11 individuals had symptoms of 5 bouts of acute tonsillitis for 2 consecutive years, compared to 39 cases that had symptoms of tonsillitis lasting more than 7 episodes every year. It is interesting to note that no child experienced 3 episodes of acute tonsillitis in 3 straight years. Therefore, it is evident that patients sought medical attention when a child had more episodes of tonsillitis than when they had a long-lasting sickness. This study showed that 40% of the patients were positive for *H. pylori* by using Giemsa staining of the tested tonsillar tissues which agrees with the study by Ahmed Abdel Aziz, [17] which had been shown that 56% of the studied patients were positive for rapid urease test and had *H. pylori* antigen in the stool. The number of acute attacks of tonsillitis per year was significantly more frequent in patients with positive *H. pylori* compared to negative ones. 40% of the patients were positive on Giemsa modification staining examination. There is a significant difference between the groups regarding bad dietary habits (consumption of fast food and restaurant food, meat, and nonfiltered water showing more positive *H. pylori* cases with the above factors. In the study by Ahmed Abdel Aziz [17], 56% of the studied patients were positive rapid urease test and had *H. pylori* in the stool. Fifty-eight percent of the patients were histopathological positive and had *H. pylori* antigen in stool. They compared with Abdel Monem, et al. [20] who conducted research on the frequency of chronic recurrent tonsillitis; utilizing RUT, they discovered that 53% of patients tested positive for *H. pylori*. The prevalence rate was lower overall than using RUT, and it was discovered in Ahmed Abdel Aziz's [17] research, of which 65% were positive for *H. pylori*. The author did not mention proton pump inhibitors or antimicrobial medications in the inclusion criteria, which could lead to false-negative results and explain the discrepancy in the results. In contrast, Singh et al. [19] found that recurrent tonsillitis had an incidence of LPR of 9% [18 instances]. Even in patients with LPR, no cases of HP (*Helicobacter Pylori*) colonization of the tonsils were found in this case series (laryngopharyngeal reflux). This research supported Siupsinskiene, et al. [21] findings that the chronic tonsillitis group had a considerably greater rate of *H. pylori* infection (56.5%). Significant associations between positive *H. pylori* findings and vocal fold and widespread laryngeal edema caused by laryngopharyngeal reflux. Furthermore, Harahap and Chrestella [22] reported that while using two types of staining in the detection of *Helicobacter pylori* in tonsillectomy specimens, it was discovered that using H&E

staining on 43 cases of tonsillectomy specimens revealed 23 patients (53%) of the tonsillectomy specimens had *Helicobacter pylori* with negative remaining 20 cases (47%) but when using Giemsa staining allowed for the detection of *Helicobacter pylori* in 29 cases (68%) of all tonsillectomy specimens with negative remaining 14 cases (32%) suggesting that Giemsa staining is more specific in detection of *Helicobacter pylori* in tonsillectomy specimens.

Conclusion

In 40% of the samples, *H. pylori* was detected using the Giemsa modification staining in the tonsillar tissue of patients with chronic tonsillitis, suggesting that treatment for *H. pylori* is necessary because it may play a part in the etiology of chronic tonsillitis in patients with positive *H. pylori* tests.

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Authors' contributions

ME wrote the manuscript and interpreted the results. TG shared in writing and in the revision of the manuscript. MA and MN shared in applying the protocol on cases and drafting the manuscript. MI and ME applied the protocol on cases, collected data, and shared in writing the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Following the Declaration of Helsinki, An approval of Research Ethical Committee of Al-Azhar University Assuit, Faculty of Medicine Under number: MSc/AZ.AST.ENT030/8/194/4/2021 On April 2021. Informed written consent to participate in the study was provided by all participants or their parents or legal guardians in case of children under 16.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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