To Evaluate the Role of *H. pylori* in Patients with Chronic Recurrent Tonsillitis

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Abstract The usual indication for surgical resection of tonsils is chronic recurrent tonsillitis. Literature also does not indicate the reason behind the fact that only part of the population suffers from recurrent chronic tonsillitis in spite being exposed to similar conditions. This was a prospective study, in which 50 tonsil biopsy samples obtained from chronic tonsillitis patients. Specimens were analysed with rapid urease broth test, HelicotecUT PLUS assay and Toluidine blue staining for presence of *Helicobacter pylori*. The age ranged from 4 to 34 years. The median age for patients with chronic recurrent tonsillitis was 9.5, 23 (46%) patients were male while 27 (54%) were female, presence of *H. pylori* by rapid urease broth test, HelicotecUT PLUS assay and Histopathology was 4%. Our analysis revealed that *H. pylori* did not significantly colonize the tonsils and does not play a role in the pathogenesis or development of chronic tonsillitis. The heterogeneity in study population and methodology may have contributed to the non significant results.

Keywords Tonsillitis · *Helicobacter pylori* · Toluidine blue · Rapid urease test

Abbreviation

H. pylori *Helicobacter pylori*
h Hours

MALT Mucosa associated lymphoid tissue
mm Millimetre
min Minutes
Ltd Limited
GABHS Group A beta hemolytic streptococcus

Introduction

Tonsillar diseases are common in paediatric and adult otolaryngological practice. Wide varieties of diseases affect this tissue often requiring tonsillectomy. The usual indication for surgical resection of tonsils is chronic recurrent tonsillitis. Literature also does not indicate the reason behind the fact that only part of the population suffers from recurrent chronic tonsillitis in spite being exposed to similar conditions [1].

Helicobacter pylori is a gram-negative, spiral shaped, urease-producing bacterium that colonizes gastrointestinal mucosa. It has a well-known role in the pathogenesis of gastric and duodenal ulcers and has also been implicated in gastric cancers, specifically mucosa-associated lymphoid tissue (MALT) lymphoma [2]. Many studies have been conducted implicating the oropharynx as a potential reservoir of *H. pylori* apart from gastrointestinal tissue, suggesting that the tonsils may also be an important reservoir for colonization. The presence of bacterial colonization on tonsillar tissue may have a role in recurrent infection of tonsillar tissues [3, 4].

The aim of this study was to assess the association between *H. pylori* colonization in recurrent infection of the tonsils. Understanding the role of *H. pylori* in the pathogenesis of chronic tonsillitis is important because it is a

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disease that is so often treated in everyday otolaryngologic practice.

Materials and Method

This was a prospective study, in which 50 tonsil biopsy samples obtained from chronic tonsillitis patients. All enrolled patients met the paradise criteria for elective tonsillectomy due to chronic recurrent tonsillitis [5].

Rapid urease test

Once tonsillectomy operation was done, one tonsil per patient was collected and two pieces of 3 mm gross specimen was cut out using a sterile blade. Use of core tonsillar tissue is recommended due to its sensitivity compared to a surface swab [6]. one specimen was then placed in a test tube containing rapid urease broth test (HiMedia laboratories Ltd, M1828-500G) and an initial colour read at 0 min. Subsequent colour changes were read at 6 and 24 h. Any colour change from the initial yellow colour to pink was recorded as positive. Any test tube that remained yellow after 24 h was recorded as negative. No readings were taken after 24 h (Fig. 1).

Second specimen was placed in HelicotecUT PLUS kit (Strong Biotech corporation) for colour change. It was observed for 1 h duration. Initial yellow colour was considered as negative and Change to Pink colour was indicative of Positive for *H. pylori* (Fig. 2).

Fig. 1 Rapid urease broth test

Fig. 2 HelicotecUT PLUS ASSAY

Any colour change is based on detection of urease, a hydrolase produced by *H. pylori*. The test system is a test tube filled with a urea containing broth where the tonsillar tissue is inoculated and allowed to incubate. Urease which is found in *H. pylori* will hydrolyse the urea in the broth. This will lead to accumulation of ammonium ion. This will then cause a rise in the pH and this is detected in the pH indicators by a colour change in the system from yellow to pink or red [7].

Histopathology

Tonsillar tissue samples were fixed in 10% formalin for 24 h, then paraffin-embedded tissue block is prepared. Procedure of Toluidine blue staining, paraffin section were cut off into 5 micrometre thickness, deparaffinize and hydrate the sections with distilled water. Then incubated with the stain for 15 min, washing well in water, dehydration, clearing, and mounting. Each slide was separately evaluated for the presence or absence of Helicobacter-like organisms at high power magnification (100×) (Fig. 3).

Results

Fifty patients were enrolled in this study. The age ranged from 4 to 34 years. The median age for patients with chronic recurrent tonsillitis was 9.5, 23 (46%) patients were male while 27 (54%) were female, presence of *H. pylori* by rapid urease broth test, HelicotecUT PLUS and Histopathology was 4% (Tables 1, 2, 3, 4). HelicotecUT PLUS test was negative in all the cases.

Discussion

Sore throat has an annual incidence of 100 in 1000 with Tonsillitis accounting for approximately 32 in 1000 patients per year. Approximately 200,000 Tonsillectomies are being performed in India every year [8]. Tonsillitis mostly affects the children below 15 years of age which

Fig. 3 Toluidine blue histopathology

Low Power Magnification 10x High Power Magnification 100x

Arrow Indicates H.Pylori

Table 1 Baseline characters

Male	23 (46%)
Female	27 (54%)
Age	11.38 (mean)

Table 2 Rapid urease broth test

Positive	1 (2%)
Negative	49 (98%)

Table 3 Histopathology by toluidine blue stain

Positive	1 (2%)
Negative	49 (98%)

increases the school absenteeism and decreases the school performance. So, detecting the aetiology will help in controlling the disease. If *H. pylori* might be a causative agent for recurrent tonsillitis, treating the recurrent tonsillitis is effective and control is achievable. Treating the chronic tonsillitis would decrease the surgical risk and improves the health status of the community.

GABHS carriage may be the cause for recurrent chronic tonsillitis because of ability of formation of biofilm in the tonsillar tissues. The antibiotic therapy may be difficult to eradicate GABHS from the tonsillar tissue. Other organisms like *S. pyogenes*, *S. aureus* and *H. influenza* also cultured from recurrent tonsillitis specimen [9, 10]. Like above mentioned organism hpylori also resides in the tonsillar tissue, so role of hpylori should be evaluated in pathogenesis of chronic recurrent tonsillitis.

Zahedi et al., showed the existence of *H. pylori* by Rapid urease test and in some histology findings potentiate the hypothesis that this bacterium might be able to live in oral cavity, where it can be regarded as one of the infection resources and may play a role in oral transmission of this common bacterium. They concluded from the studies that tonsils and adenoid tissues are the candidate places for the growth of *H. pylori* [11].

Peter et al., studied about the Prevalence of *H. pylori* in tonsillar tissue of patients with adenotonsillar hypertrophy using Rapid Urease Test in a tertiary hospital in a sub Saharan country and they concluded that Colonisation by *H. pylori* of the palatine tonsils is a new frontier with

Table 4 *H. pylori* positive results

S. No	Age	Sex	HelicotecUT PLUS	Rapid urease broth	Toluidine blue staining
1	5	M	Negative	Negative	Positive
2	12	M	Negative	Positive	Negative

conflicting results depending on the accuracy of the test method used and population studied. More studies need to be performed to ascertain the different rates of colonisation based on geographical regions [8].

The findings of published studies on the impact of *H. pylori* on the upper respiratory tract are inconsistent. Whether or not *H. pylori* is located in the upper respiratory tract and whether or not it plays a role in the pathogenesis of upper respiratory tract diseases remain unresolved [12].

Patients were not evaluated for gastro esophageal reflux disease because *hpylori* related gastritis may or may not be associated with tonsillar *hpylori* colonisation. Correlation of these two sites colonisation requires detailed analysis but our only aim is to evaluate the association of *H. pylori* in chronic recurrent tonsillitis.

For the purpose of the present study, results from Rapid urease test broth, HelicotecUTPLUS, and Toluidine blue staining were all accepted because these are widely reliable tests for gastric *H. pylori* and there is currently no accepted gold standard method of testing for the oral form [13, 14].

But few reviewed studies provide no evidence that *H. pylori* infection plays a role in the pathogenesis or development of chronic tonsillitis. Also, *Helicobacter pylori* colonization was not found to be more prevalent on tonsillar tissue with chronic recurrent infections [13].

Our data therefore suggest that *H. pylori* do not play a significant role in the infectious process leading to recurrent infections of the tonsils. *H. pylori* was found to not colonize adult tissue samples with chronic tonsillitis. Limitations to this study include small group, lack of sub group analysis and choosing a gold standard method to detect *H. pylori* in oral form.

Conclusion

Our analysis revealed that *H. pylori* did not significantly colonize the tonsils and does not plays a role in the pathogenesis or development of chronic tonsillitis. The heterogeneity in study population and methodology may have contributed to the non significant results. This study needs to be conducted in larger population with different age distribution to come for a final conclusion.

Summary

- The proper understanding of the magnitude of tonsillitis and the factors associated with the occurrence in the community is important to enable the formulation of health care services aimed at detection and treatment of tonsillitis.
- *Helicobacter pylori* colonization was not found to be more prevalent on tonsillar tissue with chronic recurrent infections
- No gold standard test available to detect *H. pylori* in oral cavity
- This study needs to be conducted in large population with different age distribution to come for a final conclusion.

Compliance with Ethical Standards

Conflict of Interest All authors declare that they have no conflict of interest.

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Informed Consent Informed consent was obtained from all individual participants included in the study.

Ethical Approval We have received ethical clearance from our institute ethical committee before starting the study.

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