

Isolation of Helicobacter Pylori in Saliva and Its Association with Upper Gastrointestinal Symptoms in Subjects with Minor Recurrent Aphthous Stomatitis – A Cross Sectional Study

¹Dr. Lavanya N, PG Student, Department of oral medicine and radiology, Government Dental College and Research Institute, Bangalore

²Dr. Vijayalakshmi KR., Professor and HOD, Department of oral medicine and radiology, Government Dental College and Research Institute, Bangalore

Corresponding Author: Dr. Lavanya N, PG Student, Department of oral medicine and radiology, Government Dental College and Research Institute, Bangalore

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Abstract

Background & Objectives: Recurrent aphthous stomatitis is the most common ulcerative disorder of oral mucosa. The etiopathogenesis of RAS is multifactorial; however recent studies have proposed *Helicobacter pylori* as one of the causative factor. *Helicobacter pylori* is a known pathogen causing upper gastrointestinal symptoms and gastric ulcers. Isolation of this microorganism from saliva is one of the non-invasive methods. This study was designed to assess the upper gastrointestinal symptoms and isolation of *Helicobacter pylori* in subjects with minor recurrent aphthous stomatitis and to investigate its association.

Methods: 60 individuals (50 minor recurrent aphthous stomatitis subjects and 10 controls) were recruited in this study. The upper GI symptoms were assessed in study

group through a detailed questionnaire. The saliva sample was collected from study subjects through spitting method. The obtained sample was inoculated on the Thayer martin medium with VCNT supplement and incubated for 3 to 5 days. The confirmation was made by typical colony formation, oxidase test and urease test. The results obtained were tabulated, statistically analyzed and observations were drawn.

Results: Isolation of *Helicobacter pylori* in saliva of subjects with minor recurrent aphthous stomatitis was possible in 5 subjects, however not able to isolate from the subjects of the control group. No statistically significant association of upper gastrointestinal symptoms with minor recurrent aphthous stomatitis and controls. No statistically significant association of *Helicobacter pylori* in saliva of

subjects of minor recurrent aphthous stomatitis and controls.

Conclusion: The present study observed that no significant association of *Helicobacter pylori* and upper gastrointestinal symptoms in minor recurrent aphthous stomatitis.

Keywords: Recurrent aphthous stomatitis, *Helicobacter pylori*, upper gastrointestinal symptoms, saliva.

Introduction

Recurrent aphthous stomatitis (RAS, aphthae) is a very common yet a poorly understood mucosal disorder that is characterized by multiple recurrent small, round, or ovoid ulcers with circumscribed margins, erythematous haloes, and yellow or gray floors.^{1,2}

The estimated point prevalence of oral ulcers worldwide is 4%, aphthous ulcers, affects as many as 25% of the population worldwide. The peak age of onset is between 10 and 19 years, it may continue throughout the entire human lifespan.³ These ulcers are often accompanied by significant pain, disproportionate to the size of these lesions, although they usually heal within 10–21 days, it presents as a public health burden which may lead to a negative impact on the patient's quality of life, irrespective of its etiology.^{4,5}

Although the etiology is multifactorial, local and systemic factors have been suggested to affect the development of RAS. Some of the predisposing factors include trauma, hormonal changes, diet, nutritional deficiencies, Celiac disease, and immunological disorders and genetic polymorphisms.⁶ Several studies have investigated the role of microorganisms which includes Bacterial (*Streptococci*, *H. pylori*, *Lactobacillus*) Viral (*Varicella zoster*, *Cytomegalovirus*) in the etio- pathogenesis of RAS, and few of the studies have shown the possible involvement of *H. pylori* infection in individuals with RAS, but their relationship remains debatable. *H. pylori* is a

microaerophilic, gram-negative spiral bacterium that infects more than 50 % of human gastric mucosa.⁷ The prevalence of *H. pylori* infection is high (49.94%-83.30%) in India, due to poor socioeconomic status, poor hygiene, inadequate sanitation conditions, overcrowding, consumption of contaminated water and food, and bacterial infection within the household.⁸ *H. pylori* plays a critical role in the etiopathogenesis of chronic superficial gastritis, peptic ulcer disease, gastric adenocarcinoma, and mucosa-associated lymphoid tissue lymphoma.^{7,9} *H. pylori* related upper GI symptoms are heartburn, subconsciously rubbing the chest, gastric reflux and belching.¹⁰

The similar histological features between RAS and peptic ulcers, supports the association between RAS and *H. pylori* infection.⁷ *H. pylori* has been detected in gastric secretions, faeces, saliva, dental plaque of healthy individuals and also in patients with upper digestive system diseases.¹¹ Detection of *H. pylori* involves invasive and non-invasive methods which includes culture, rapid urease test (RUT), and histopathological analysis of the biopsy tissue, serological examination of blood and the urea breath test (UBT). It has also been recovered from saliva.¹² Isolation of this pathogen from saliva is a non-invasive method which may be beneficial in the management of RAS by their eradication.

Aims

- I. To assess the symptoms of Upper Gastrointestinal diseases and to isolate and identify the *Helicobacter pylori* by culture of saliva in subjects with minor recurrent aphthous stomatitis.
- II. To study the association of *Helicobacter pylori* and upper gastrointestinal symptoms in subjects with minor recurrent aphthous stomatitis.

Methodology

This study was conducted on 60 subjects of minor RAS and 10 age and sex matched controls who were selected

based on the selection criteria during the period of December 2016 to June 2018 visiting Department of Oral Medicine and Radiology, Government Dental College and Research Institute, Bangalore. The study was conducted in full accordance with ethical principles and was reviewed and approved by an ethical board of the institution. All the selected subjects were informed about the details of the study in their known local language and a written informed consent was obtained. Symptoms of upper gastrointestinal diseases was recorded as per Yamamichi et al¹³ following which a thorough intraoral examination of the oral cavity with the emphasis on the features of RAS.

Inclusion Criteria For Cases

1. Subjects with clinically diagnosed minor recurrent aphthous stomatitis. (Based on the WHO criteria).
2. Subjects with the age group between 18-60 years.

Inclusion Criteria for Control Subjects

Subjects visiting the Department of Oral Medicine and Radiology, Government Dental College and Research Institute fulfilling the following criteria:

1. Age and sex matched subjects with that of the study group.
2. Subjects without history of recurrent aphthous stomatitis, salivary gland disease or history of treatment for H pylori infection in the past 6 months

Exclusion Criteria

1. Seropositive patients with oral ulcers, Patients having oral ulcers with autoimmune diseases (behcet's disease, reiters syndrome etc.,) or Patients taking drugs like proton pump inhibitors, H2 receptor blockers, or Non-steroidal anti-inflammatory drugs, or any drugs that can alter salivary composition and quantity.
2. Patients with the history of antibiotic usage in past 1 month, or salivary gland disorders or other mucosal lesion of oral cavity, History of deleterious habits such as

smoking, alcoholism, Pregnant and lactating women, history of allergies. The whole unstimulated saliva (WUS) was collected by navazesh method.¹⁴ A selective medium – Thayer martin agar plates supplemented with vancomycin, colistin, nystatin and trimethoprim lactate (VCNT) (110x40 mm{HI Media laboratories, Mumbai} (stored between 20C-80C) were inoculated with saliva samples with the inoculated loop dipped in saliva. The culture plates were incubated at 37 degrees Celsius in a micro aerophilic environment in the incubator for 5 to 7 days.

The presence of *Helicobacter pylori* was confirmed by microaerophilic growth, and identified by their morphology – greyish translucent colonies checked at the interval of 5th and 7th day. Firstly, it was checked on 5th day for the colonies, if no colonies were formed, it was subsequently checked on the 7th day of incubation. Further it was confirmed through oxidase and urease test.

Results

All the 60 cases were in the age range of 18 to 60 years with mean age of 28.94 ± 8.644 years. Among 60 cases, 48 were males and 12 were females. The ulcers more frequently occurred in labial mucosa - 27 (48.2%) followed by vestibule – 11 (16.0%), buccal mucosa - 9 (18.0%), lateral border of tongue- 2 (2.0%) and ventral surface of the tongue - 2 (10.0%), dorsal surface of tongue - 3(6.0%).(Graph 1) The number of ulcers ranged from 1 to 3 in number, out of which 32 (64.0%) subjects had single ulcer, 12 (24.0) subjects had two ulcers,6(12.0%) subjects had three ulcers during the time of presentation.

H/O heartburn symptom, was present in 29 (58.0%) of cases and 5 (50.0%) of controls, **H/O bloating** symptom was present in 24(48.0%) of cases and 5(50.0%) subjects of controls. **H/O stomach feeling heavy after meals** was present in 14(28.0%) of cases and 4(40.0%) of controls, **H/O sometimes subconsciously rubbing chest with the**

hand symptom was present in 9(48.0%) and 2(20.0%) subjects of controls. **H/O heartburn after meals** symptom was present in 14 (28.0%) of cases and 4(40.0%) subjects of controls. **H/O feeling full while eating meals** - This symptom was present in 22(44.0%) of cases, 3 (30.0%) subjects of controls. **H/O burping a lot** This symptom was present in 14 (28.0%) of cases and 3(30.0%) subjects of controls.(Graph 2 and 3)

The Association between *H pylori* isolation in cases and controls - *H pylori* isolation was positive in 5(10%) subjects of RAS group whereas all the subjects were negative (.0%)in control group. Overall Cases had higher proportion of subjects with *H pylori* isolation, compared to controls. The differences in the *H pylori* isolation between cases and controls was not statistically significant (P value -0.296) (Graph 4)

Discussion

The worldwide distribution, high frequency and decreased quality of life associated with RAS have resulted in extensive research into the etiology and efficient therapy towards the disease. However, the etiology of RAS still remains unclear, and the currently available therapy remains inadequate. *H pylori* has been isolated from various specimens by invasive and noninvasive methods; its isolation from saliva is one of the noninvasive modality. However, the results of most of the studies conducted are contradictory.

In our study, out of the 50 subjects with RAS, 40 (80.0%) were male subjects and 10 (20.0%) were female subjects indicating a male predominance contradicting the many published reports by **Donatsky et al**,¹⁵ **Axe'll et al**¹⁶ which shows female predominance. However, **DiazGuzman et al**,¹⁷ **Hidalgo F Rivera et al**,¹⁸ **Katti et al**,¹⁹ **Kareem et al**,²⁰ **Rao et al**,²¹ **Shrivastava et al**²² reported higher male predilection at point prevalence and

female predilection at annual prevalence,¹⁸ which was similar to the point prevalence noted in our study.

Association between *H pylori*, recurrent aphthous stomatitis and upper gastrointestinal symptoms:

Many studies have addressed the relationship between infection with *H. pylori* and the presence of RAS and its association. The samples employed for *H pylori* isolation are saliva, biopsy or swab from the lesion. We employed the method of isolation of *H pylori* from saliva, similar to **Ferguson et al**.¹² In our study *H pylori* was isolated from five subjects (10%) with RAS and none from the control group. The age of the subjects varied between 20-40 years, with male predominance. RAS and *H pylori* commonly affects younger age group which was also noted in our study. The prevalence of *H pylori* is higher in developing countries, with most of the infections occurring during 2nd and 3rd decade of life, and they seem to be decreasing with improvements in hygiene practices, as poor hygiene and crowded conditions may facilitate transmission of *H pylori* infection.²³ Among the subjects in whom *H pylori* was isolated the distribution of upper GI symptoms were- 4 (13.8%) subjects had H/O heartburn, 4(16.7%) subjects had H/O bloating, 2 (14.3%) subjects had H/O stomach feeling heavy after meals, 3(21.4%) subjects had H/O heartburn after meals, 3 (13.6%) subjects had H/O feeling full while eating meals, 1(6.7%) subject had H/O burping a lot.

Guzik et al²⁴ reported the presence of *H pylori* in saliva in 53.2% of study subjects without concurrent gastric infection.

Thus **Guzik et al**²⁴ had extended an opinion that *H pylori* can infect any of the patients, but it can aggravate the symptoms in patients with oral lesions.

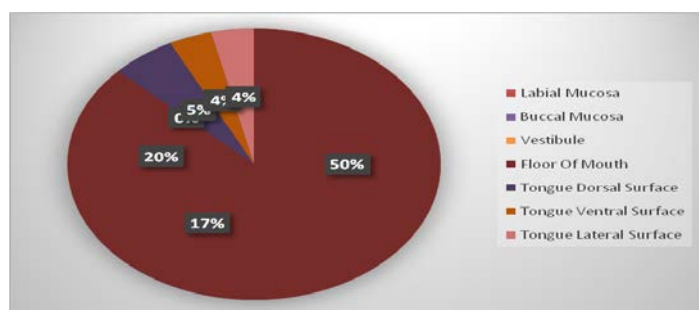
Farmaki et al.²⁵ in his study reported that most of the patients with RAS were *H pylori* positive confirmed by ELISA test of serum and saliva.

Karaca et al.²⁶ reported the presence of bacteria in gastric mucosa in 65% of RAS subjects.

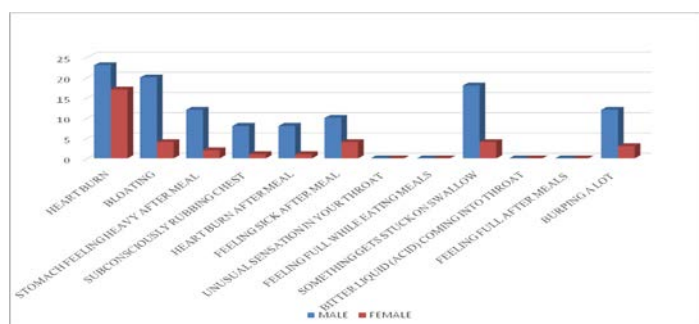
However, Shimoyama et al.²⁷ employed culture method for isolation of H pylori by obtaining the swab from the ulcer surface, their results showed that none of patients were positive for the bacteria in the culture.

Chapman et al.²⁸ did not find any presence of H. pylori in the biopsy samples obtained from patients with active RAS and history of RAS after performing the culture.

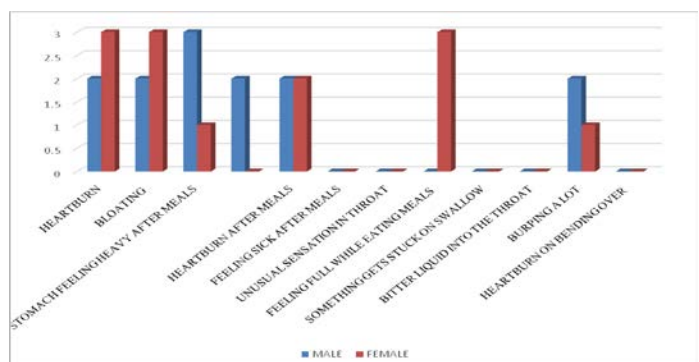
Graph 1: Showing the distribution of ulcers in different intra oral sites:



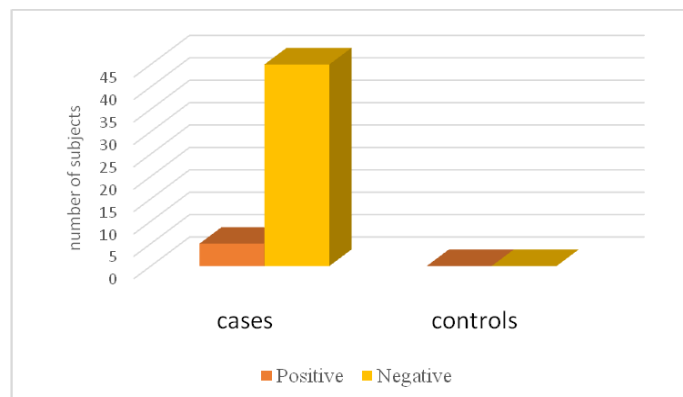
Graph 2: showing the distribution of upper GI symptom among cases:



Graph 3: showing the distribution of upper GI symptom among controls:



Graph 4: Showing the distribution of H pylori isolation in cases and controls:



Conclusion

The results of our study suggest that statistically no significant association (p value -of upper gastrointestinal symptoms and H pylori with that of minor recurrent aphthous stomatitis. Hence the study with larger sample size and more sensitive method to isolate this pathogen is required.

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