ABSTRACT

Candida spp. were found in the gastric mucosa of 8 (8.9%) patients, out of whom 7 (7.3%) showed coexistence of the fungi with H. pylori. Analysis of relationship between selected disorders of the upper gastrointestinal tract (nonulcer dyspepsia NUD, gastric ulcer, duodenal ulcer) and infection with H. pylori and/or Candida revealed a link between coexistence of H. pylori with Candida and gastric ulcers suggesting synergism of those microorganism in pathogenesis of the disease.

Keywords: Helicobacter pylori, Candida species.
INTRODUCTION

Infection with *Helicobacter pylori* (*H. pylori*) is one of the most widespread bacterial infections all over the world. An estimated 50% of world human population is infected with this bacterium. The incidence of the infection is associated mostly with childhood, socioeconomic and sanitary conditions. In developing countries the incidence accounts for 80-100% while in developed countries 20-40%. According to the studies carried out in 1995-1996 by Goscińskiak, *H. pylori* strains were significantly more frequent in adults than in children (57.4% and 38.1% respectively).

*H. pylori* plays important role in ethiopathogenesis of the upper gastrointestinal tract disorders. The species is etiological factor of type B gastritis and one of significant risk factors for ulcerous disorder, gastric lymphoma MALT (Mucosa Associated Lymphoid Tissue) and gastric carcinoma. Chronic gastritis develops in all persons infected with *H. pylori*. However, further development of pathological changes occurs in only part of them. Ulcerous disease (gastric ulcer in 70% of cases and duodenum ulcer in 90% of cases) develops in some 15% of infected patients while proliferation of a neoplasm in the stomach occurs in 2-5% of them. The role of *H. pylori* in the stomach carcinogenesis was proved in an animal model (Mongolian gerbils) [1,2]. Intensity of pathological changes in the stomach is influenced by the host characteristics (genetic predispositions, intensity of the immune response to infection, diet, life conditions) as well as the level of strain toxicity. In patients infected with low-virulent *H. pylori* strains the development of mild gastritis transforming to the chronic form is more probable while in persons infected with more virulent strains digestive ulcers, atrophy of the gastric mucosa and even neoplasmatic changes can occur. High attention is now paid to the role of *Candida* fungi in chronic gastritis, ulcerous disease and non-specific inflammation of the bowel. Fungal colonization of the gastric mucosa was shown to be present in 30-50% of patients with active ulcerous disease. Persistency of clinical symptoms is prolonged and the process of ulcer healing is affected. Moreover, the strains isolated from gastric ulcers were demonstrated to show greater proteolytic activity than those isolated from chronic gastritis patients [3]. In most of healthy persons yeast-like fungi of the *Candida* genus, as a natural saprophytic flora, inhabit the oral cavity, pharynx and large intestine areas in an equilibrium with gastrointestinal bacteria. The esophagus, stomach and small intestine stand only for a way of passage for them. In high-risk patients persistent fungal colonization can contribute to the development of pathological changes. Fungal infection of the gastrointestinal tract is usually endogenous. Exogenous infection is also possible from solid food contaminated with fungi. Recently, attention has been drawn to the fact that *H. pylori* infection can represent a factor facilitating fungal colonization of gastric mucosa [4,5]. Low pH in stomach is assumed to be not a barrier to survival and pathogenic action of *Candida* fungi. *In vitro* studies showed the ability of *C. albicans* and *C. tropicalis* to grow in an environment of pH = 2 and *C. lusitaniae* at pH = 3. Which is in line with observations of other authors who reported cases of patients with gastric ulcers localized in the prepyloric area in whom concurrent high fungal colonization at very low pH was found. This phenomenon suggests that there exists not fully investigated efficient mechanism adapting *Candida* fungi to survive at low pH that occurs in the stomach [6,7].
The aim of the study presented in this paper is:

1. To analyse the co-existence of *H. pylori* and *Candida* fungi infections in patients with clinical symptoms from the upper gastrointestinal tract.

**MATERIALS AND METHODS**

The study was conducted with the approval from the institutional Ethical Committee, SBMCH, Chennai-44. Permission to conduct the study was sought from the respective hospital authorities. Informed consent was obtained from the patients before the enrolment into the study.

This is a prospective cross sectional study conducted over a period of eighteen months from May 2012 – October 2013.

This study was carried out at The Department of Microbiology in collaboration with The Department of Surgical Gastroenterology, The Department of Medical Gastroenterology, The Department of Pathology, SBMCH, Chennai-44.

**STUDY GROUP**

Outpatients and inpatients, of both sexes in the age group 20 – 70 years, based on the following criteria were included in the study.

**Inclusion criteria**

- Patients with complaints suggestive of upper gastro intestinal diseases in Alcoholic and Non-Alcoholic Gastritis.
- Patients with antral gastritis, duodenitis, gastric ulcer and duodenal ulcer.
- Patients who were not on antibiotics, proton pump inhibitor or Helicobacter eradication therapy within 1 month prior to inclusion in this study.

**Exclusion criteria**

- Patients with previous gastric surgery.
- Patients with active bleeding.
- Gastric carcinoma.

**Specimen Collection and Transport**

**Biopsy Sample**

 Patients fasted overnight before endoscopy. Endoscopy was done using fiber optic endoscope. The endoscope and the biopsy forceps were rinsed thoroughly with water and soaked in 2% gluteraldehyde for 20 minutes and were thoroughly rinsed with sterile normal saline just before the collection of specimen.
Four biopsy samples were taken from the antrum (2 cm from the pylorus) and were transferred to respective Eppendorf tube under sterile conditions. One sample was inoculated into urea broth for rapid urease, two specimens were transported in normal saline for culture, Gram stain and Giemsa stain and the last specimen was placed in 10% formalin for histopathological examinations.

The specimens for culture were transported to the laboratory and were inoculated on the culture media without delay.

**Processing of Specimens**

**Rapid Urease Test**

An antral biopsy tissue was placed in an Eppendorf tube containing 1 ml of Rapid urease test broth (HiMedia RUT broth M1828, prepared as per the manufacturer’s instruction. Colour change from yellow to pink at room temperature within two hours, were taken as positive.

**Culture**

Biopsy tissue was crushed between two sterile glass slides and the minced tissue was inoculated onto freshly prepared campylobacter agar base with 5% defibrinated sheep blood and Campylobacter Selective Supplement and chocolate agar (non selective media). The plates were incubated at 37°C in a candle jar with a pad of cotton soaked in water placed at the bottom. The plates were examined for bacterial growth between three to seven days. Characteristic small, translucent circular colonies were confirmed by gram stain, catalase, oxidase and urease. Antimicrobial sensitivity was performed by Disc diffusion method using commercially available antibiotic discs.

**Confirmatory tests for suspected colonies**

**Gram stain**- Gram negative curved bacilli were seen.

**Oxidase test** - The suspected colony was streaked on the surface of oxidase disc containing 1% tetramethyl paraphenylene diamine dihydrochloride. An intense purple colour developed within 5 seconds and was recorded as positive. Positive and negative controls were used.

**Urease test** - The colony was emulsified in 0.5 ml of the urea broth. An instant colour change from yellow to pink was noted as positive.

**Catalase test** - The suspected colony was introduced with a glass rod into 3% hydrogen peroxide taken in a clean test tube. Immediate production of gas bubbles was noted as positive. Positive and negative controls were also tested.

**Crush cytology**

Another biopsy tissue was crushed between two sterile glass slides and the minced tissue was used to make to two smears.
Gram stain

One of the slides was air dried and heat fixed. The slide was covered with methyl violet for one minute, excess stain was poured off, Grams iodine was added and washed after 1 minute. This was followed by acetone for 2-3 seconds. The acetone was washed and the slide was counter stained with dilute carbol fuschin for one minute, washed with water, blotted dry and observed under oil immersion objective. H. pylori appeared as gram negative curved bacilli.

Giemsa stain

The other slide was air dried and fixed with methanol for 3 minutes, 2-3 drops of undiluted Giemsa stain was added and kept for 5 minutes. The smear was then washed with water, blotted dry and seen under oil immersion objective. The organism appeared deep purple with the typical gull-wing morphology.

Histopathology

One specimen was fixed in 10% formalin, paraffin sections were made and stained with Haematoxylin and Eosin (H&E) and examined for H. pylori.

Candida Species were identified by Gram staining, Sabouraud’s dextrose agar culture and germtube test.

RESULTS

Endoscopy was carried out in 109 (100%) persons with symptoms from the upper gastrointestinal tract. Infection with and presence of H. pylori in biopsies collected from gastric mucosa was confirmed in 90 (82.6%) of the persons examined.

Candida fungi were found in 8 (8.9%) patients. Co-existence of H. pylori and Candida in the stomach was found in 7 (7.3%) patients.

The incidence of duodenal ulcer was greater in the group infected with H. pylori alone as compared to the group infected neither with H. pylori nor Candida. Gastric ulcers were significantly more frequent in the group with concurrent H. pylori and Candida infections.

Table 1

<table>
<thead>
<tr>
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<th>No. of Positive Cases</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>H. pylori</td>
<td>90</td>
<td>82.6%</td>
</tr>
<tr>
<td>Candida Species</td>
<td>8</td>
<td>8.9%</td>
</tr>
</tbody>
</table>

Coexistence of H.pylori and candida infection seen in 7 (7.3%) of the cases.
DISCUSSION

In the study a preliminary attempt to demonstrate importance of colonization of gastric mucosa with *Candida* in pathogenesis of gastric and duodenal disorders was made. It is worth emphasizing that colonization means the presence of fungi in material collected from patients in the quantity not pathologically significant and resulting neither in infection nor in clinical symptoms of fungal infection. Studies carried out by Scott and Katzenstein confirm growing number of such cases (16% and 33% respectively) [8,9]. This phenomenon seems to be alarming as colonization may lead to fungal infection not only in persons of high-risk group in whom favorable factors occur but also in healthy persons [10]. In our study *Candida* fungi were revealed in the stomach of 8 (8.9%) patients with ailments from the upper gastrointestinal tract. However, in all patients the fungi counts were not pathologically significant (<10³ CFU/ml). Those patients cannot be deemed as having mycosis, however, the presence alone of fungi in the stomach, even in low quantity, may cause injury in epithelium and changes in mucosa as a result of action of proteolytic enzymes produced by fungal blastoconidia or spores [10]. In present study the importance of fungi presence in gastric mucosa was analyzed in the context of their co-existence with *H. pylori* that may result in intensification of inflammatory changes [11]. Co-existence of *Candida* with *H. pylori* was proved in 7 (7.3%)

CONCLUSIONS

- Demonstrated relationship between co-existence of *H. pylori* with *Candida* (in non-significant quantity) and gastric ulcer may confirm their synergistic action and impact on pathogenesis of that disease, but this issue needs further studies.
- A link between the presence of *Candida* without concurrent *H. pylori* and incidence of gastric or duodenal ulcer was not proved.
- Fungal colonization in upper gastrointestinal tract diseases should be evaluated in pre and post eradication of Helicobacter pylori infection.

REFERENCES