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The Possible Role of *Helicobacter pylori* in the Development of Sjogren's Syndrome and Chronic Sialadenitis

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Background: Sjogren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration of exocrine glands, which can be triggered by environmental factors such as viral infection. Chronic obstructive sialadenitis is the most common type of chronic sialadenitis and many different bacterial infections develop as a result of ductal obstruction.
Objectives: This study was conducted to assess the association of these lesions with the presence of *Helicobacter pylori*.
Patients and Methods: A total of 56 biopsies diagnosed as Sjögren's syndrome (SS) and chronic sialadenitis (CS) due to sialolithiasis in submandibular glands, sublingual and minor salivary glands were selected (56 samples as examined group and 20 samples as control group). All the paraffin blocks were cut for hematoxylin and eosin (H and E) staining to confirm the diagnoses and then the samples were prepared for immunohistochemistry (IHC) staining to detect *H. pylori*. Chi-squared test was used for statistical analysis.
Results: Chi-squared test showed a significant difference between *H. pylori* positivity in the groups examined (P = 0.046) and between SS group and normal tissue samples (P = 0.013). There was no significant difference between gender and *H. pylori* positivity in examined groups examined (P = 0.574, P = 0.543, respectively). In addition, there was no significant difference between gender and *H. pylori* positivity in SS group (P = 0.119, P = 0.331, respectively) also in CS group (P = 0.981, P = 0.571).
Conclusions: Bacterial infection has been suggested in the pathogenesis of both SS and CS. In addition, *H. pylori* is a resident of the oral cavity, thus may be involved in the development and progression of these lesions. Hence, search for *H. pylori* antibody in blood of patients with SS is suggested.

*Keywords:*Sjogren's Syndrome; Chronic Sialadenitis; *Helicobacter pylori*; Oral Cavity

1. Background

Sjogren's syndrome (SS) is a chronic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands. It affects salivary glands and associated with autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematous and systemic sclerosis (1). SS is one of non-infectious causes of salivary gland tissue inflammation (2). Patients with SS suffer from progressive dryness of eyes and mouth due to insufficient salivary and lacrimal secretions (3). In histological examination, characteristic features of salivary glands can be seen including destruction of the acinar structure, total replacement of the salivary acinar structure by lymphocytic infiltrate, and also various changes in ductal structure such as epimyoepithelial islands which can be seen mostly in parotid gland tissue (2-4). According to previous studies, sensitivity and specificity of the parotid biopsy is comparable with that of labial biopsy in diagnoses of SS (4). In addition, patients with SS have an increased risk of developing gastric lesions, such as gastric atrophy (5). Previous studies showed chronic gastric inflammation in 80% of patients with SS (6). In histopathological examinations, epithelial and glandular alterations are similar to those of gastritis with *H. pylori* infection (7). Chronic obstructive sialadenitis is the most common type of CS (8). Periductal lymphocytic infiltration, acinar atrophy and periductal fibrosis are the histological features of chronic obstructive sialadenitis. Sialolithiasis is the most common cause of chronic obstructive sialadenitis and many different bacterial infections develop as a result of ductal obstruction (2). *H. pylori* is a gram negative, spiral shaped organism, which colonizes in human gastric mucosa. The association of *H. pylori* with pathogenesis of peptic ulcers, gastric adenocarcinoma and low-grade B-cell mucosa-associated lymphoid tissue lymphoma has been proved (9).

Many studies indicated that *H. pylori* can be isolated from the oral cavity, the dorsum of the tongue, salivary secretions and dental plaque (supragingival plaque and subgingival plaque) in patients with or without periodontitis (10, 11). *H. pylori* exists in the oral cavity even

after systemic eradication therapy, suggesting that patients with positive results for gastric *H. pylori* have *H. pylori* in the oral cavity as well. Particularly in cases with gingivitis or periodontitis, the oral cavity can be considered as a reservoir capable of increasing the risk of gastric re-infection (12). The presence of *H. pylori* in some other oral lesions has been reported. For example, in patients with burning, halitosis and lingual dorsum hyperplasia, 87% of patients had positive results for *H. pylori* (13). In addition, in atrophic glossitis, benign migratory glossitis and burning mouth syndrome, *H. pylori* was detected in 16% of patients (14). In one study, *H. pylori* was detected in patients with aphthous stomatitis in 52% of cases (15). In another study, 38.9% of patients with recurrent aphthous stomatitis showed *H. pylori* positivity (16). Previous studies showed that SS was triggered by environmental factors such as a viral infection (17). infection has also been also considered as an etiological factor in developing SS and CS due to sialolithiasis (2).

2. Objectives

This study was conducted to assess the association of these lesions with the presence of *H. pylori*.

3. Patients and Methods

A total of 56 biopsies diagnosed as Sjögren's syndrome (SS) and chronic sialadenitis (CS) due to sialolithiasis in submandibular, sublingual and minor salivary glands and 20 tissue samples taken from the labial mucosa of the lower lip and floor of mouth (as submandibular and sublingual salivary glands excretory ducts drain to the floor of mouth) with pathology report as "without significant pathological changes" were selected as the control group from the archive of Pathology Department of Loghman Hospital, Tehran, Iran. All the paraffin blocks were cut for H&E staining to confirm the diagnoses and then the samples were prepared for IHC staining.

In brief, formalin-fixed, paraffin-embedded tissues were all cut into 4 μm. The slides were then deparaffinized, rehydrated and pre-treated with trypsin for 40 minutes at 37°C according to manufacturer's instructions (Novocastra, UK). The endogenous peroxidase activity was blocked, followed by incubation with lyophilized rabbit polyclonal antibody (Novocastra, UK) at a dilution of 1: 20 for one hour. DAB was used to visualize the complex. Finally, the sections were counterstained with haematoxylin and mounted. *H. pylori* positive and negative human gastric samples served as positive and negative controls, respectively. Statistical analysis was performed using SPSS version 21.0.1, Chi-squared test and independent-samples T test were used. Significance was set at $P < 0.05$.

4. Results

In this study, there were 34 (44.7%) male and 42 (55.3%) female. In general, the ages of patients and control group ranged from 19 to 70 years, with a mean age of 38.83 years. Demographic characteristics of samples and *H. pylori* detection status is summarized in Table 1. Chi-squared test showed a significant difference between *H. pylori* positivity in the groups ($P = 0.046$) and between SS group and normal tissue samples ($P = 0.013$). There was no significant difference between gender and *H. pylori* positivity in examined groups ($P = 0.574$, $P = 0.543$, respectively). In addition, there was no significant difference between gender and *H. pylori* positivity in SS group ($P = 0.119$, $P = 0.331$, respectively) and in CS group ($P = 0.981$, $P = 0.571$). Independent-Samples T test showed a significant difference between SS and CS groups and age ($P = 0.002$).

Table 1. Demographic Characteristics of Samples and *H. pylori* Detection Status

Type of Lesion	No. of Patients	Mean Age Range, y	*H. pylori* Positivity [a]
Sjogren's syndrome	25		18 (72)
Male	5	45.40	
Female	20	27-70	
Chronic sialadenitis	31		17 (54.8)
Male	20	33.19	
Female	11	19-46	
Normal tissue	20		7 (35)
Male	9	39.35	
Female	11	20-70	

[a] Data are presented as No. (%).

5. Discussion

Exposure of bacteria to host tissues results in some pathologic outcomes. Several immune effectors function to minimize microbial interactions with host tissues (bacterial-epithelial contact). There is growing evidence from animal models indicating that certain bacteria can trigger immunopathology (18). The association between infection and autoimmune diseases has been found previously (19).

Focal lymphocytic infiltration around the ducts is the first histopathological feature in newly diagnosed SS cases suggesting that antigen presentation of glandular or acinar epithelium has a crucial role in SS pathogenesis (20). Epithelial cells produce locally different B-cell targeted cytokines, and B-cell activating factor (BAFF) (21). Previous investigations indicated that proinflammatory cytokines such as IL-1 and IL-6 are produced by epithelial cells and lymphocytes in SS, which strongly suggest that both epithelial cells and lymphocytes (autoimmune epithelialitis) are important to initiate SS (22). On the other hand, T-cell CD4 lymphoid hyperplasia in SS contributes to B-cell hyperactivity due to exogenous or autoantigens. In addition, B-cell activation is another finding in patients with SS. Thus, in SS, T cells in the salivary glands must be stimulated by antibodies, which enhance B cell proliferation (23). Growing evidence indicates that patients with

SS have an increased risk of lymphomas as well as gastric lesions (5). In the current study, 72% of SS samples, 54.8% of CS samples and 35% of normal tissues showed *H. pylori* positivity in IHC staining (Table 1). The present study also demonstrated a statistically significant difference in *H. pylori* positivity between SS tissues and normal tissue samples. Figure 1 shows the presence of *H. pylori* in both SS and CS samples.

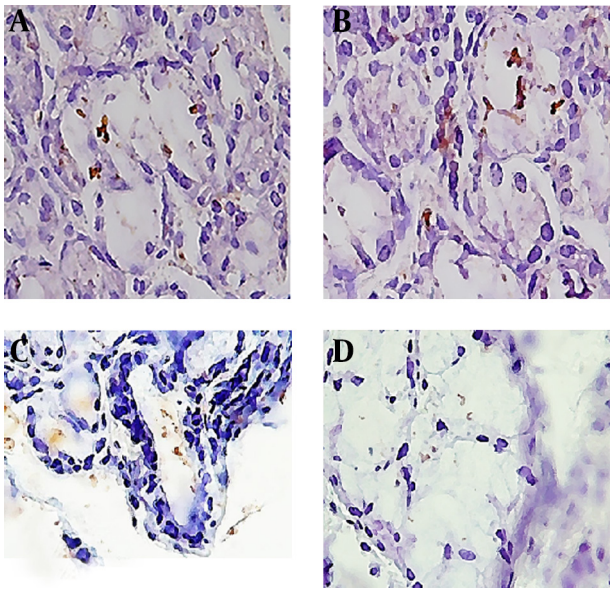

Figure 1. Presence of *H. pylori* in Both Sjogren's Syndrome and Chronic Sialadenitis Samples

In a study on patients with SS (primary and secondary) carried out by El Miedany et al. it was shown that prevalence of *H. pylori* infection and average titers of anti-*H. pylori* antibodies were significantly higher compared to patients with connective tissue diseases or healthy control subjects. Due to a high prevalence of *H. pylori* infection in the studied group, the authors considered a significant correlation between *H. pylori* infection and disease duration, and suggested that the longer the duration of SS, the greater the likelihood of having *H. pylori* infection in patients (24). Aragona et al. working on patients with different autoimmune diseases showed that 79.4% of patients with primary SS had antibodies against *H. pylori* and there was also a significant difference between the SS group and patients with various autoimmune diseases regarding *H. pylori* serum antibody (25). Banno et al. found *H. pylori* antibody in 75.5% of SS patients. There was also a significant difference between the level of *H. pylori* antibody in patients and control groups (26).

Previous investigations demonstrated that *H. pylori* can produce extracellular products that cause local and systemic immune responses resulting in tissue damage (27). *H. pylori* infection induces a humoral response which may contribute to surrounding tissues damage (28). *H. pylori* pathogenesis acts through two mechanisms. Firstly, *H. pylori* interaction with surface epithelial cells develops direct cell damage or produces pro-inflammatory mediators (29). Secondly, *H. pylori* reaches the underlying mucosa, hence stimulates immune response, which in turn leads to liberation of different cytokines and oxygen radicals (30). The presence of *H. pylori* in the lamina propria has been proven indicating *H. pylori* invasion to the underlying tissues, induction and development of inflammation (31). In addition, *H. pylori* was detected inside the blood vessels, which may explain *H. pylori* bacteremia and systemic responses (32). Ito et al. found that *H. pylori* is able to pass through the endothelial layer (31). Endothelial cell injury may induce vasculitis, which results in the release of inflammatory mediators and systemic immune response (33). Within *H. pylori* infected gastric mucosa, IL-8 is increased (34). CD4+ T cells migrate to gastric mucosa and cause epithelial damage through proliferation, apoptosis and metaplasia (35). This might be caused by a direct bacterial effect like IL-8 or as a consequence of cellular immune response (36). CD4 T cell absence in *H. pylori* associated gastritis leads to increased gastritis dominated by an infiltration of CD8 T cells (37). Thus, it seems that CD8 T cells contribute to *H. pylori*-induced pathology. *H. pylori* antigen-presenting cells have interactions with CD4-expressing cells, which bind to B-cells in the marginal zone leading to T-cell activation, lymphoid follicle formation, and B-cell proliferation in gastric mucosa and therefore development of gastric lymphoma (38).

Patients with SS also have over a 44-fold increased risk of development of B-cell non-Hodgkin's lymphoma (39). There is some evidence of gastric and parotid MALT lymphoma regression after *H. pylori* eradication (40). It was suggested that possible mechanisms for infection could be direct infection of salivary gland tissue by *H. pylori* or recirculation of organism related antigens from another site of infection (41). Taken together, it is suggested that during *H. pylori* related MALT lymphomas, there is a close interaction among epithelial cells, T cells and B cells.

In conclusion, bacterial infection has been suggested in the pathogenesis of both SS and CS. In addition, *H. pylori* is a resident of theoral cavity, thus can be involved in development and progression of these lesions. It is suggested to search for the *H. pylori* antibody in the blood of patients with SS. Regarding the high risk of MALT in patients with SS, which had regressed after *H. pylori* eradication, eradication of *H. pylori* in these patients can be helpful for patient survival, especially in the early stages of SS.

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