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Detection of *Helicobacter pylori* in Oral Lichen Planus and Oral Lichenoid Reaction

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Background: Oral Lichen Planus (OLP) is a chronic inflammatory disease affecting the oral mucosa in 0.5-2% of the world's population. It is more common in women compared to men and the mean age at the onset of the lesion is the fourth decade.
Objectives: The purpose of this study was to evaluate the presence of *Helicobacter pylori* (*H. pylori)* in Oral Lichen Planus and Oral Lichenoid Reaction.
Materials and Methods: A total of 41 biopsies diagnosed as Oral Lichen Planus and Oral Lichenoid Reaction and 15 samples as the control group were selected from the archives of Pathology Department of Loghman Hakim Hospital, Tehran, Iran from 2002 to 2009. All the paraffin blocks were cut for hematoxylin and eosin (H and E) staining to confirm the diagnoses and the samples were then prepared for immunohistochemistry (IHC) staining. Statistical analysis was performed using SPSS statistical software (version 21.0), the chi-squared test and Fisher's exact test, and independent-samples t test. Statistical significance between the groups was set at $P < 0.05$.
Results: The *H. pylori* positivity was found in 29.7% and 14.8% of OLP, and OLR samples, respectively. Statistically significant difference was not observed compared to normal tissues ($P = 0.661$). The chi-squared test show no significant difference between the frequency of *H. pylori* positivity and the lesion type, gender, and site. Although *H. pylori* positivity was found in 59.2%, and 50 % of OLP, and OLR samples, respectively, statistically significant difference was not observed compared to normal tissues ($P = 0.838$). In addition, the chi-squared test show no significant difference between the site of the lesion and *H. pylori* positivity. *H. pylori* positivity was mostly found on the buccal mucosa (64.3%), however, *H. pylori* negativity was mostly found on the tongue (60 %) ($P = 0.309$). Additionally, the chi-squared test show no significant difference between the frequency of *H. pylori* positivity, and the gender ($P = 0.517$). Independent-samples t test showed no statistically significant difference between age and two patient groups statistically ($P = 0.450$).
Conclusions: This present study reveals no significant difference between the presence of *H. pylori* in OLPs and OLRs. Yet, further studies with larger sample size needs to be done to prove this association.

Keywords:Helicobacter pylori; Oral Lichen Planus; Immunohistochemistry

1. Background

Oral Lichen Planus (OLP) is a chronic inflammatory disease affecting the oral mucosa in 0.5-2% of the population. It is more common in women compared to men and the mean age at the onset of the lesion is the fourth decade (1). The etiology of OLP is not clear, but genetic background, dental materials, drugs, bacterial and viral infections, autoimmunity, immunodeficiency, food allergies, stress, habits, trauma, diabetes and hypertension, malignant neoplasms, bowel diseases have been suggested as the etiological factors (2-4). Immune dysregulation plays a critical role in the pathogenesis of OLP (3). Growing evidence indicates that some endogenous or exogenous factors might initiate the cell mediated immunity (5). Oral lichenoid reaction (OLR) is considered as a variant of OLP. It is suggested that OLR is a disease by itselfIt can also be considered as an exacerbation of an existing OLP due to some medications, or as an allergic reaction to dental materials (6). OLP and OLR share common clinical and histological features (6, 7). The World Health Organization (WHO) histopathological criteria are: presence of a well-defined band-like infiltration of lymphocytes, signs of liquefaction degeneration in the basal cell layer, and absence of epithelial dysplasia (8). *Helicobacter pylori* (*H. pylori*) is a gram negative, spiral shaped organism that colonizes in human gastric mucosa. It has been found mostly in developing countries probably due to poor sanitary conditions (9). *H. pylori* is one of the most common bacteria colonizing the human gastrointestinal (GI) tract (10). The association of *H. pylori* with the pathogenesis of peptic and duodenal ulcers has also been proven (11). Previous studies have proven the presence of *H. pylori* in the oral cavity. In addition to the dental plaque and saliva, it can be found in other oral sites as well as some oral lesions such as oral ulcers (9, 12, 13).

2. Objectives

The aim of this study was to evaluate the presence of *H. pylori* in OLP and OLR. If the association between the development of OLP and OLR and the presence of *H. pylori* could be found, eradication therapy against *H. pylori* instead of a long term corticosteroid therapy in cases of OLP might be more effective and safer.

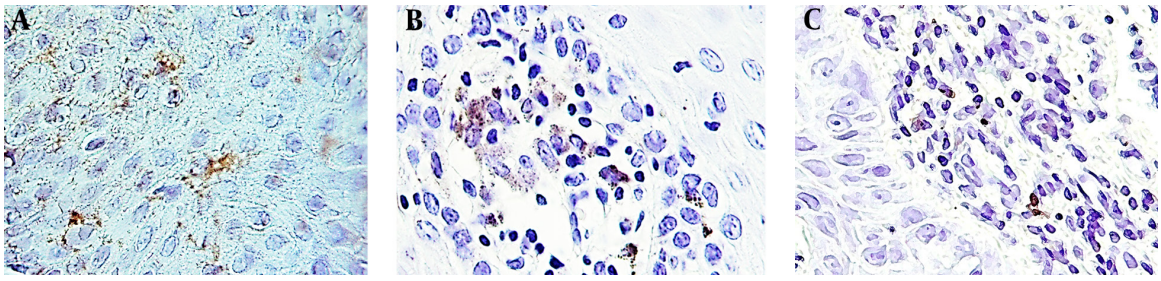
3. Materials and Methods

A total of 27 biopsies diagnosed as OLP and 14 samples of OLR were selected from archives of the Pathology Department of Loghman Hakim Hospital, Tehran, Iran from 2002 until 2009. Fifteen tissue samples taken from different areas of the oral cavity for other purposes, such as crown lengthening, and also samples with pathology reports stating "without significant pathological changes" were selected as the 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 the iimmunohistochemistry (IHC) staining. Briefly, 4-μm-thick sections of paraffin-embedded formalin-fixed specimens were cut. The slides were deparaffinized, rehydrated and pre-treated with trypsin for 40 minutes at 37°C according to the manufacturer's instructions (Novocastra, UK). The endogenous peroxidase activity was blocked by hydrogen peroxide, followed by incubation with lyophilized rabbit polycolonal antibody (Product Code: NCL-HPp Novocastra) at a dilution of 1:20 for one hour. DAB was used to visualize the complex. Then, the sections were counterstained with hematoxylin and mounted. *H. pylori*-positive and -negative human gastric samples were used as positive and negative controls, respectively. All sections were assessed by Olympus microscope at 400× magnification by two pathologists independently. Statistical analysis was performed using SPSS version 21, the chi-squared test, Fisher's exact test, and independent-samples t test. Statistical significance between the groups was set at $P < 0.05$.

4. Results

In this study, there were 22 males (39%) and 34 females (61%) (including normal samples). In general, the ages of the patients ranged from 21 to 85 years, with a mean age of 46.91 ± 13.73 years. Demographic characteristics of the samples and *H. pylori* detection status are summarized in Table 1. Although *H. pylori* positivity was found in 28.57 %, and 12.5 % of OLP, and OLR samples, respectively, nostatistically significant difference was not observed compared to normal tissues ($P = 0.838$). In addition, there was no significant difference between OLP and OLR groups regarding *H. pylori* positivity ($P = 0.571$). The chi-square test showed no significant difference between *H. pylori* positivity and the site, and gender in two patient groups $P = 0.697$, $P = 0.529$, $P = 0.242$, respectively. Table 2 shows the presence of *H. pylori* in different areas of the oral cavity. Although the chi-squared test show no significant difference between the site of the lesion and *H. pylori* positivity; *H. pylori* positivity was mostly found on the buccal mucosa (64.3%), however, *H. pylori* negativity was mostly found on the tongue (60 %) ($P = 0.309$). In addition, the chi-squared test ($P = 0.517$), and Fisher's Exact test ($P = 0.354$) show no significant difference between the frequency of *H. pylori* positivity and the gender. Additionally, independent-samples t test did not show any significant difference between the age and two patient groups ($P = 0.450$) (Figure 1).

Figure 1. Sections of Oral Mucosa Immunostained With *H. pylori* Antibody



A) In the normal epithelium; B) In Oral Lichen Planus section; C) In Oral Lichenoid Reaction section (× 400).

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

Type of Lesion	Gender, No.	Age Range	Number of Patients	*Helicobacter pylori* Positivity, No. (%)
Oral Lichen Planus			27	16 (59.2)
Male	9	29-60		
Female	18	24-85		
Lichenoid Reaction			14	7 (50)
Male	7	33-64		
Female	7	28-61		
Normal tissue			15	8 (53.3)
Male	6	21-47		
Female	9	23-67		

Table 2. Summary of *Helicobacter pylori* Detection in Different Regions [a]

Normal Tissue	Oral Lichen Planus		Lichenoid Reaction		Normal Tissue	
***H. pylori* status**	+	-	+	-	+	-
Buccal mucosa	9	5	5	4	4	1
Tongue	5	4	0	2	1	3
Gingiva	2	2	2	1	3	3
Total	16	11	7	7	8	7

[a] Data are presented as No.

5. Discussion

In this study, the presence of *H. pylori* in normal oral tissue and oral lesions; OLP and OLR were reviewed using IHC. In the present series, *H. pylori* positivity was detected in 16 cases of OLP, 7 cases of OLR, and 8 cases of the normal tissue. Authors did not find a similar study in the literature, thus, comparing the results with of other studies was limited. In a study on upper GI in patients with OLP, Sanli et al. found *H. pylori* positivity in 9/20 of the patients with both OLP and chronic atrophic gastritis (14). Attia et al. in a study on 20 samples of upper gastrointestinal and erosive OLP detected the presence of *H. pylori* in 9 patients (45%) with erosive OLP. However, they did not find any association between OLP and *H. pylori* induced gastritis (15). It might be due to lack of a significant association between these two diseases. Taghavi Zenouz et al. using urea breathing test, observed 80% of *H. pylori* positivity in patients with OLP. However, 66.7% of the control group also were positive. They did not find any significant difference regarding *H. pylori* positivity between the groups examined (16). Pourshahidi et al. using blood samples, detected the *H. pylori* antibody in 21/41 of OLP patients and 51/82 healthy samples. They did not observe any significant difference between the examined groups. However, they assumed that a higher percentage of *H. pylori* antibody positivity in the control group might be due to high *H. pylori* infection in the population (17). Recently, when the current work was under review, another article on OLP and its association with *H. pylori* was published. In this article, 10 normal samples from the buccal mucosa were compared to 50 samples of OLP and 10 gastric biopsies regarding *H. pylori* positivity by employing IHC. None of the normal buccal and OLP samples showed *H. pylori* positivity, however, *H. pylori* was detected in all gastric samples (18). Riggio et al. carried out a study on 20 biopsies of OLP taken from buccal and labial mucosa and floor of mouth along with 10 normal gingival samples. Using Polymerase Chain Reaction, *H. pylori* was not detected in none of the above mentioned samples (19). Different results from the current work can be explained by the variations in sample size, geographic variation, and the technique used for detection. There are some published reports regarding the effects of *H. pylori* eradication in patients with Lichen Planus (LP). For example, Vainio et al. observed a higher frequency of peptic ulcer in the patients with chronic or recurrent LP, compared to the patients with a transient LP and the control group. In addition, they found *H. pylori* infection in 66% of the patients with chronic or recurrent LP, and suggested that eradication therapy should be useful in those patients (20). It has been proposed that *H. pylori* reaches with surface epithelial cells and causes cell damage or produces pro-inflammatory mediators. In addition, *H. pylori* reaches the underlying mucosa to stimulate immune response leading to the liberation of different cytokines (21). *H. pylori* can disrupt the gastric epithelial cells which leaves the underlying basement membrane bare (22). After invasion, *H. pylori* is engulfed by gastric epithelial cells (23). Later, *H. pylori* binds to the basement membrane laminin of the gastric mucosa (24). There is a growing research evidence of the invasion of *H. pylori* to the lamina propria of the gastric mucosa. The presence of bacteria in the lamina propria indicates a true invasion (23). A high rate of cell turnover may expose extracellular matrix components such as laminin for the bacterium (25). Fan et al. indicated an increase in the number of $CD8^+$ in the lamina propria in *H. pylori* positive samples compared to negative ones, but there was not any significant difference in the number of $CD4^+$ T lymphocytes between the samples (26). Both $CD4^+$ T helper cells and $CD8^+$ cytotoxic T cells are also activated in OLPs. Previous studies showed that adjacent to damaged basal keratinocytes, T cells are mostly $CD8^+$ cytotoxic T cells. However, the number of $CD4^+$ T cells does not increased in the areas of basement membrane disruption. Chemokines may be released by activated $CD8^+$ cytotoxic T cells and possibly keratinocytes which attract other immune cells (27). In addition, in *H. pylori* infected gastric mucosa, mast cells infiltrate between epithelial cells, and the number of mast cells decreases after eradication of *H. pylori*. On the other hand, mast cells degranulate in *H. pylori* infected gastric mucosa. These findings may prove the role of mast cells in the pathogenesis of *H. pylori* infection in chronic gastritis (28). The number of mast cells also increases in OLPs. Approximately 60% of the mast cells are degranulated in OLPs. Thus, mast cells might be involved in the pathogenesis of OLPs (29). Jose et al. in a study on the role of mast cells in OLPs and OLRs,

found a significant increase in the number of mast cells in both lesions. The authors suggested an important role of the mast cells in the pathogenesis of both lesions. They also showed a marked degranulation of mast cells in the sub-epithelial zone (30). Another study carried out by Jahanshahi et al. showed the dense infiltration of the mast cells beneath the band like lymphocytes, or attached to the vessels (31).

Song et al. found the highest *H. pylori* distribution in molar teeth at 82%, followed by the premolar area and anterior teeth at 64% and 59%, respectively (32). This might explain the higher frequency of OLPs and OLRs in the buccal mucosa, tongue, and gingiva (33-35). Additionally, Holmstrup et al. found that local factors such as dental plaque and calculus worsen gingival OLPs (36). Mignogna et al. suggested an adequate control of plaque and calculus in OLP patients for periodontal health maintenance (34). These findings can prove the role of microorganisms in the development of OLP. In conclusion, the present study did not indicate a significant difference between the presence of *H. pylori* in OLPs and OLRs, probably due to the small sample size or high *H. pylori* infection in the population. In addition, high percentage of *H. pylori* positivity in the control group might prove the high percentage of contamination in Iran as it is more prevalent in developing countries. Yet, further studies with larger sample sizes need to be done to prove this connection.

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