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Probable Association Between Oral Lichen Planus and presence of *Helicobacter Pylori*: A Preliminary Study in a Chilean Population

Probable Asociación entre Liquen plano Oral y la presencia de *Helicobacter pylori*: Estudio Preliminar en Chile

Sebastián Araneda[1,2,3]; Christian Castillo[1]; Bernardo Venegas[2] & Ulrike Kemmerling[1]

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ABSTRACT: Oral Lichen planus (OLP) is one of the main inflammatory diseases of the oral mucosa that is considered as a potentially malignant disorder. The exact pathogenesis of OLP remains to be completely understood. However, presence of bacteria has been associated to the inflammatory response observed in OLP. Particularly, *Helicobacter pylori* a major etiological agent of gastrointestinal inflammatory diseases and risk factor for gastric cancer, has been associated to Lichen planus. Here we studied a group of Chilean patients if there is any association between the presence of *Helicobacter pylori* and the clinical manifestation of OLP. We found a significant difference between the patients positive for *H. pylori* and the age of OLP diagnosis, suggesting that oral *H. pylori* might induce the disease at an earlier age. However, we could not confirm a statistically significance between the presence of the bacteria and OLP.

KEY WORDS: *Helicobacter pylori*, Oral Lichen planus

INTRODUCTION

Lichen planus (LP) is a common chronic inflammatory condition that affects skin and mucous membranes. Particularly oral lichen planus (OLP) is one of the main inflammatory diseases of the oral mucosa (Alrashdan el al., 2016). While cutaneous lesions of LP can be self-limiting and pruritic, oral lesions are mostly chronic, non-remissive and can be a source of morbidity. The precise etiology of OLP is far from being completely understood, moreover only few predisposing factors are currently thought to potentially have a role in its pathogenesis (Alrashdan *et al*.; Parashar, 2011).

Clinically, six clinical subtypes of OLP are described that can be observed individually or in combination: reticular, plaque-like, atrophic, erosive/ulcerative, papular and bullous (Alrashdan *et al*.; Gorouhi *et al*., 2014). However, some authors classify OLP into 2 broad groups: 1) Predominately white lesions, typically of hypertrophic papilar and reticular ones and 2) Predominately red lesion that are associated to atrophic, eritematous, erosive or ulcerative ones (Gandolfo *et al*., 2004; Ismail *et al*., 2007) (Fig. 1).

The histopathological features of OLP is characterized by liquefaction of the basal layer of epithelia, band-like lymphocytic infiltration at the lamina propia, and degenerating keratinocytes (Choi *et al*., 2016). The lymphocytes present in OLP are mainly CD4+ and CD8+ T cells, and CD8+ T cells are supposed to mediate the destruction of the oral epithelial cells (Choi *et al*.; Gorouhi *et al*.).

[1] Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile, Chile.
[2] Facultad Ciencias de la Salud, Universidad de Talca, Chile.
[3] Facultad de Odontología, Universidad San Sebastián, Chile.
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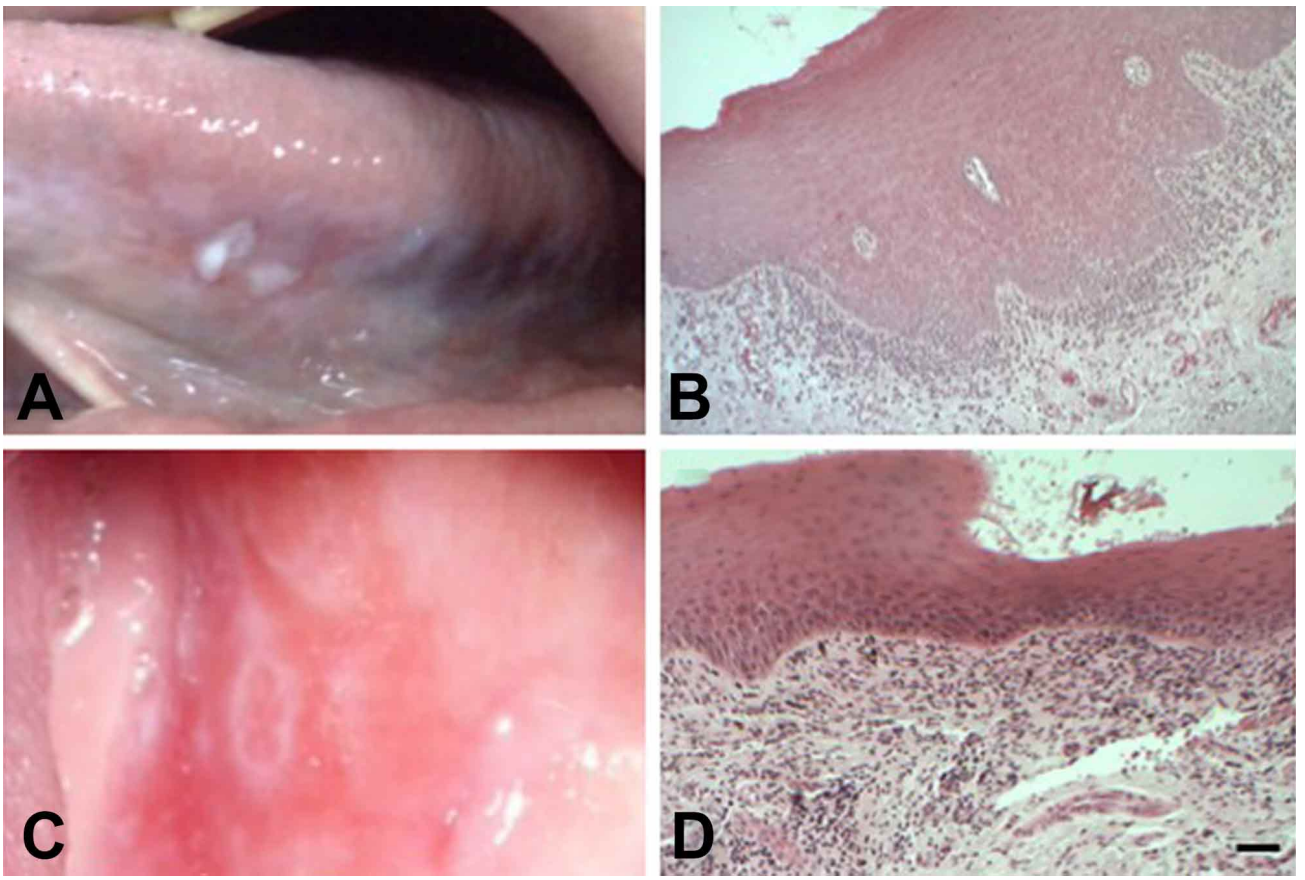

Fig. 1.Clinical and histopathological characteristics of OLP: Panel A and B shows hypertrophic and panel C and D shows atrophic variants of OLP, respectively. (A) Hypertrophic OLP are predominately white lesions, and can be reticular, papular or plaque-like. (B) Representative imagen of a hyperplasic OLP is shown: Epithelial hyperplasia, hydropic degeneration of the basal cells and inflammatory infiltrate arranged in juxtaepithelial band can be observed. (C) Atrophic OLP are predominantely red lesions and can be erythematous, erosive, ulcerated and bullous. Reticulated lesions might be present. (D) Representative imagen of an atrophic OLP is shown: Atrophic epithelium accompanied by inflammatory infiltrate arranged in juxtaepithelial band. (B) and (D) Samples were processed for routine histopathological analysis, scale bar: 20 mm.

Bacterial invasion has been related to OLP, being considered as trigger or aggravating factors since infection of the oral mucosa stimulates the inflammatory cells (Choi *et al*.).

Helicobacter pylori (*H. pylori*) is a common bacteria that affects more than 50 % of the world population (Attia *et al*, 2010). *H. pylori* is a slow-growing, microaerophilic, gram-negative bacterium and constitutes a major etiological agent of chronic gastritis, gastric and duodenal ulcers and is also a risk factor for gastric cancer (Amiri *et al*., 2015; Song *et al*., 2000). Importantly, it has been proposed that the bacterium is associated with dermatological conditions, including LP (Attia *et al*.; Loster *et al*., 2006). Moreover, the oral cavity is now considered as an ecological niche for *H. pylori* (Attia *et al*.). Since OLP is as an oral potentially malignant disorder (OPMD) (Warnakulasuriya, 2018) and considering that particularly the oral ulcerative/atrophic lesions present an higher potential of malignant disorder (Casparis *et al*., 2015), here we studied a group of Chilean patients if there is any association between the presence of *H. pylori* and the clinical manifestation of OLP.

MATERIAL AND METHOD

Sample selection: Twenty-eight samples of OLP were obtained from the Oral Diagnostics Clinic at the "Facultad de Ciencias de la Salud, Universidad de Talca", between the years 2008-2016. Two samples of healthy oral mucosa from two voluntary donor patients were also obtained. The study was approved by the Ethics Committee of the "Universidad de Talca" (2017-05-BV-

SA); all participating subjects signed an informed consent form. Data about age and sex was obtained from the clinical record (Table I).

Histopathology: All samples were processed by conventional histological methods. Briefly, the formalin-fixed tissues were dehydrated in alcohol, clarified in xylene, embedded in paraffin, and sectioned at 5mm. Paraffin-embedded histological sections were stained with hematoxylin-eosin (SigmaAldrich, USA) for routine histological analysis. The patient samples were diagnosed into atrophic-hypertrophic according to the biopsy report and were additionally revised for the presence of dysplasia (Table I).

DNA amplification: Genomic DNA was extracted from the paraffin-embedded samples using a commercial kit (NucleoSpin Tissue; Machery-Nagel) according to the manufacturer's instructions and quantified by QUBIT Fluorometric System (Invitrogen®). For *H. pylori* DNA amplification, a sequence of 109 base pairs of the bacterial 16S gene rRNA was amplified using the primers: H.pyl-Fw (5'-CTGGAGAGACTAAGCCCTCC-3') and H.Pyl-Rev(5'-ATTACTGACGCTGATTGTGC -3')(Chong *et al*., 1996). Purified *H. pylori* DNA was used as positive control (a kind gift from Dr. Héctor Toledo, Instituto de Ciencias Biomédicas, Facultad de Medicina, Universidad de Chile). 1 ng of total DNA of each sample was amplified using SaphireAMP Fast PCR Master Mix (Takara, Japan) according the following cycling program: denaturation of 95 °C for 5 minutes, followed amplification by 40 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds and 72 °C for 30 seconds. The elongation was carried out at 72 °C for 10 minutes. PCR products were subject to electrophoresis in 2% agarose gels and stained with GelRed (Biotium, USA). PCR markers from 100bp Sharp DNA marker (ATZ labs) were employed as molecular weight standards.

Table I. Data of patients with OLP between 2008-2016 from the clinical record at the "Clinica de Diagnóstico Oral, Universidad de Talca": F: female. M: male. (+): present or registered. (-): negative or not registered.

#	Age	Sex	Hypertrophic OLP	Atrophic OLP	Dysplasia	*H. pylori*
1	61	F		X	-	-
2	54	M	X		+	-
3	-	F		X	-	+
4	54	M		X	+	-
5	47	F		X	-	+
6	64	M	X		+	-
7	-	M	X		+	+
8	54	F		X	-	-
9	52	F	X		-	-
10	51	F		X	+	-
11	42	F		X	-	-
12	38	F		X	-	+
13	59	F		X	-	-
14	63	F		X	-	-
15	63	F		X	-	+
16	46	F	X		-	-
17	-	-		X	-	-
18	53	F	X		-	-
19	65	M		X	-	-
20	68	F		X	-	-
21	25	F	X		+	+
22	46	F		X	-	+
23	31	M	X		-	-
24	51	F		X	-	-
25	54	M		X	-	-
26	60	F	X		-	-
27	64	F		X	-	-
28	40	F		X	+	+

Statistics: The qualitative analysis of *H. pylori* in samples of OLP were performed by Fisher's exact test through SPSS software (IBM SPSS, Nueva York, USA). It was considered a $p < 0.05$ as statistically significant.

RESULTS

Twenty-eight patients diagnosed with OLP were included in the study, 20 (83 %) and 7 (17 %) of them were female or male, respectively; evidencing a trend towards the female sex. The average age for OLP diagnosis was 52 years. One sample was excluded from this analysis for not presenting the data in the clinical record (Table I). The atrophic histopathological variant was diagnosed in 19 cases (68 %), while the hypertrophic one was reported 9 cases (32 %). The most common site of the OLP lesion was cheek (48 %), followed by the tongue (30 %), inferior gingiva and upper lip (7 %, each), and upper gingiva and lower alveolar ridge (4 %, each).

In 8 samples, DNA of *H. pylori* could be detected (29 %) (Fig. 2); 6 of the *H. pylori*-positive samples correspond to atrophic OLP (75 %) and the other 2 samples were identified in hypertrophic OLP (25 %). The patients were categorized into 2 sections according their age: under 47 and over 47. Three cases were excluded from this analysis because they did not present required data. Oral *H. pylori* positive patients manifest OLP a younger age than oral *H. pylori* negative

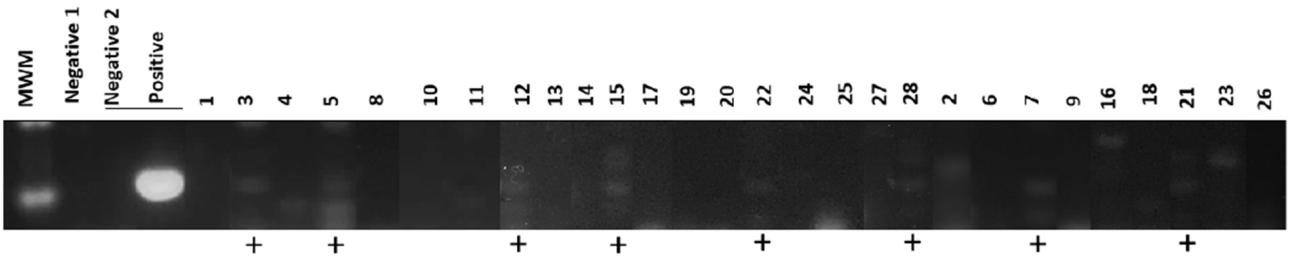

Fig. 2. Detection of *H. pylori* DNA in samples of OLP by PCR: a 109-base pair fragment of 16S rRNA gen of *H. pylori* was amplified as described in material and methods. MWM: molecular weight marker, Negative control 1 and 2 of normal oral mucosa. Positive control of DNA *H. pylori*. Lanes 1-28: atrophic OLP. Lanes 2-26: hypertrophic OLP. (+) positive samples to *H. pylori* DNA.

patients (p=0.03). Additionally, the presence of dysplasia was evaluated in the different OLP samples reported in the original biopsy reports (Table I). Only 3 of the 8 samples positive for *H. pylori* DNA were diagnosed with epithelial dysplasia.

DISCUSSION

OLP represents 2,1% of the oral mucosal lesions studied in the Chilean population (Espinoza, 2003). The exact etiology is unknown, but it is considered as an immunologic disorder of CD8+ lymphocytes (Payeras *et al*., 2013). Some studies proposed that the presence of bacteria and its tissue invasion into the oral mucosa stimulate the inflammatory cells. Choi *et al.* proposed a pathogenic model for OLP in which, similarly to the etiology of biofilm-associated gingivitis, bacteria infiltrate oral mucosa and lead chronic inflammation in host. Bacterial invasion promotes the chemotaxis of CD4 + and CD8 + LT, which produce the liquefaction of the basal stratum of the epithelium, contributing to its dysfunction. If this infection persists, the inflammation becomes chronic (Choi *et al*.).

Some of the bacteria that are increased in patients with OLP are the periodontopathic *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia* and *Treponema denticola*, suggesting that these bacteria might be irritating factors in this environment (Ertugrul *et al*., 2013). Also, an increase in *Fusobacterium nucleatum, Eikenella corrodens* and *Treponema denticola* was evidenced in the oral epithelium of patients with OLP compared to samples of the healthy mucosa of patients without OLP. These studies conclude that a disequilibrium in the oral microbiota might be responsible of alteration in the oral mucosa and point out a combination of periodontal therapy associated with antibiotics to treat OLP (Choi *et al*.). Similarly, it has been proposed that periodic periodontal treatment should be necessary in patients with *H. pylori* in order to reduce or eliminate its ecological niches, and reduce the inflammation induced by the periodontal biofilm (Souto & Colombo, 2008).

On the other hand, *H. pylori* has been detected in gastric and oral mucosa in patients diagnosed with erosive OLP. A study showed, that biopsies of gastric and oral mucosa oral from the same patients were positive for *H. pylori*, concluding that the concomitant presence of *H. pylori* in erosive OLP and in the gastric mucosa implies a possible pathogenic connection between this bacterium and erosive OLP (Attia *et al*.). Other study showed a greater presence of *H. pylori* in patients diagnosed with OLP. The detection method used was PCR of samples taken from the periodontal pocket of these patients. They analyzed 72 patients diagnosed with OLP, of whom 17 were positive for *H. pylori*. These patients also presented greater periodontal inflammatory signs, increase in depth at periodontal probing, and greater accumulation of bacterial plaque (Kazanowska-Dygdaa *et al*., 2016). Therefore, a possible fecal-oral or oral-oral transmission route of *H. pylori* should be considered. In addition, this therapy aims to prevent gastric infection of *H. pylori*, and also prevent gastric re-infection in patients who have been eradicated of *H. pylori* in the stomach (Amiri *et al*.).

In the present study we found a significant difference between the patients positive for oral *H. pylori* and the age of OLP diagnosis, suggesting that oral *H. pylori* might induce the disease at an earlier age. Therefore, patients younger than 47 years with oral *H. pylori* are more likely to develop early OPL, than people over 47 years. However, we could not confirm a

statistically significance between the presence of the bacteria and OLP, probably due to the low patient number.

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RESUMEN: Liquen Plano Oral (LPO) es una enfermedad inflamatoria de la mucosa oral considerada como desorden potencialmente maligno. La patogénesis exacta de LPO es desconocida. Sin embargo, se ha asociado la presencia de bacterias como responsables de la inflamación observada en LPO. Particularmente, *Helicobacter pylori* (*H. pylori*), agente etiológico principal de enfermedades inflamatorias gastrointestinales y factor de riesgo de cáncer gástrico, ha sido asociado con LPO. Se estudió la posible asociación entre *H. pylori* y manifestaciones clínicas de LPO en un grupo de pacientes Chilenos. Se encontró diferencia significativa entre los pacientes positivos para *H. pylori* y la edad de diagnóstico de LPO, sugiriendo que *H. pylori* podría inducir la enfermedad a temprana edad. Sin embargo, no se pudo confirmar significancia estadística entre la presencia de esta bacteria y la presencia de displasia en LPO.

PALABRAS CLAVE: *Helicobacter pylori*, liquen plano oral.

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Corresponding author:
Sebastián Araneda
Facultad de Odontología
Universidad San Sebastián
Bellavista 7
Santiago
CHILE

Email: saranedar@docente.uss.cl

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