

LINGUA PLICATA ASSOCIATED WITH INCREASED LEVEL OF PROINFLAMMATORY CYTOKINES IN GINGIVAL CERVICAL FLUID: POSSIBLE NEW SIGN IN ULCERATIVE COLITIS

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Received: 12.01.2021.

Accepted: 17.02.2021.

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ABSTRACT

Ulcerative colitis (UC) is a complex disease in which the interaction of genetic, environmental, and microbial factors drives chronic intestinal inflammation. Gastrointestinal symptoms are predominant including pathological manifestations in the oral cavity, as well as extra-intestinal complications.

Lingua plicata (LP) is a condition characterized by an increased number of fissures and grooves at the central and lateral aspects of the dorsal surface of the tongue. LP is usually asymptomatic and discovered incidentally, but the accumulation of food in the fissures and grooves can lead to focal glossitis and halitosis.

In this study, we analyzed the level of proinflammatory cytokines in gingival crevicular fluid (GCF) and demonstrated that proinflammatory cytokines IFN- γ , IL-12, and IL-1 β were significantly increased in the group of UC patients when compared with healthy controls. Obtained results have shown that 35% of UC patients have lingua plicata. The GCF levels of IFN- γ and IL-12 were higher in UC patients with LP compared with UC patients without lingua plicata.

In conclusion, increased GCF values of IFN- γ and IL-12, in UC patients with LP may be considered as a sign of the disease progression and, consequently, of a poor prognosis for patients.

Keywords: *Lingua plicata, gingival crevicular fluid, ulcerative colitis, oral cavity.*

DOI: 10.2478/SJECR-2021-0006



INTRODUCTION

Inflammatory bowel disease (IBD) comprises a group of diseases that affect the gastrointestinal tract. The pathogenesis of ulcerative colitis (UC), the form of IBD, is still unrevealed, but the contribution of immune cells in the UC development and progression is well documented. (1-3). Gastrointestinal symptoms are predominant including pathological manifestations in the oral cavity, as well as extra-intestinal complications. The main oral manifestations of UC include labial swellings with vertical fissures, pyostomatitis vegetans, angular cheilitis, perioral erythema, and glossitis (4).

There is a limited number of studies demonstrating oral manifestation and oral comorbidities of ulcerative colitis. There was an attempt to organize that information and current and adopted classification is divided between oral signs and symptoms (5).

Oral signs are vegetative pyostomatitis (pyostomatitis vegetans), aphthous ulcers, coated tongue, and periodontitis, while the symptoms are bad breath (halitosis) and impaired sense of taste (5). Since oral manifestations were not given much importance, it has not been completely clarified whether they are a consequence of disease or therapy (5, 6). Oral manifestations are more frequent and serious in the advanced stages of the disease (5). In the acute phase of ulcerative colitis, over 50% of patients have bad breath (7). Vegetative pyostomatitis is a rare occurrence in healthy people, and it is very common in patients with ulcerative colitis (8). These lesions are observed as multiple white and yellow bumps which, due to the dynamics of the oral environment, quickly burst and erode (9). Histologically, eosinophilic and neutrophilic infiltrates are observed (9, 10). The most commonly affect the hard and soft palate, buccal mucosa, the vestibular surface of the gingiva, and are least commonly detected on the floor of the oral cavity and tongue (11, 12). Aphthous ulcers are the most common oral manifestation of ulcerative colitis (13). The appearance of ulcers may or may not accompany the acute nature of the disease, they are painful and uncomfortable for sufferers and usually last about two weeks (13, 14).

Patients with UC are more likely than healthy subjects to show signs of periodontitis (15). By monitoring the depth of periodontal pockets and the loss of epithelial attachment of teeth, it was found that patients with UC are more likely to suffer from periodontitis than patients with Crohn's disease (15, 16). Studies have shown that UC patients are more likely to show symptoms of periodontal disease, which is generalized, while inflammation is less intense compared to subjects who do not have ulcerative colitis (17). Moreover, published studies have shown that over 50% of UC patients have bad breath compared to healthy subjects (15, 16). About 20% of patients report an impaired sense of taste, while patients with pancolitis have a sour taste in the mouth (15).

Lingua plicata (LP) is a condition characterized by an increased number of fissures and grooves at the central and

lateral aspects of the dorsal surface of the tongue (18). There is often a midline groove with smaller, symmetrical radiating fissures (18). This idiopathic condition is more common in older people and is thought to be a reactive process (18, 19). LP is usually part of Down's syndrome, Cowden's syndrome, and pachyonychia congenita. It is also one of three pathognomonic symptoms of Melkersson-Rosenthal syndrome (20-24). There is a recorded case of LP after chemotherapy and radiotherapy for Hodgkin lymphoma (25). LP is usually asymptomatic and discovered incidentally, but the accumulation of food in the fissures and grooves can lead to focal glossitis and halitosis. The lesions may improve spontaneously, but good oral hygiene with gentle brushing of the tongue can reduce complications. Avoiding tobacco, alcohol, and foods that irritate the mucosa of the tongue would be other general measures that should be presented to the patient (26).

While the true etiology of LP remains unknown, the histopathology of this disorder is different from that of the healthy tongue. The pathophysiological characteristics of LP include intraepithelial and subepithelial infiltrates of polymorphonuclear leukocytes and lymphocytes (27).

This study aims to show that the oral cavity is a mirror that reflects the underlying UC and to evaluate oral symptoms as indicators for assessing gastrointestinal disorders. For this purpose, we investigated whether the development of LP corresponds to the increased serum levels of inflammatory cytokines, as the sign of on-going inflammation and/or aggravation of UC.

MATERIAL AND METHODS

Patients

The study was conducted at the Center for Gastroenterology, Clinical Center of Kragujevac, Serbia, Department of Dentistry, Faculty of Medical Sciences, University of Kragujevac and Center for Molecular Medicine and Stem Cell Research, Faculty of Medical Sciences, University of Kragujevac, Serbia. All of the patients gave their informed consent and the research project was approved by two Ethics Committees of these institutions. Additionally, adherence was made to the Principle of Good Clinical Practice and the Declaration of Helsinki at all times.

We investigated a total of 20 patients with ulcerative colitis (12 males and 8 females; mean age: 48 ± 2.99 years). The control group consisted of 13 healthy individuals. Control subjects were selected from volunteers at the Department of Dentistry. The diagnosis of ulcerative colitis was based on endoscopic and histopathological criteria. The patients with ulcerative colitis were classified into two groups, according to the diagnosis of lingua plicata.



Gingival crevicular fluid sampling and biochemical measurements

Gingival crevicular fluid (GCF) samples were collected from the eight teeth of patients with confirmed UC diagnosis and healthy control. The samples were retrieved 24 hours after the colonoscopy. The study included patients with no previously diagnosed periodontal pockets, sulcus with a maximal depth of 4mm, and patients with no previously oral treatment. The samples were retrieved using the filter paper technique. In brief, the sampling site was isolated, air dried, and isolated with cotton rolls, the supra-gingival biofilm was gently removed and then a fine sterile paper strip (Periopaper, Pro Flow, Amityville, NY, USA) was inserted into the peri-periodontal sulcus until mild resistance was felt and left in place for the 30s. Strips that were visually contaminated with blood or saliva were discarded. The paper strips were then inserted in micro-centrifuge plastic tubes containing 0.5 ml of sterile phosphate-buffered saline. Following 10s of vortexing, the samples were centrifuged for 5 min at 1500 g to separate the cells and debris and then the paper strips were removed (28). The obtained samples were stored at -20°C until being processed for biochemical analysis by enzyme-linked immunosorbent assays (ELISA).

Determination of cytokines in gingival fluids

The ELISA assay was conducted according to the handbook provided by the ELISA kit (R&D Systems, Minneapolis, MN, USA). The optical density of each sample at 450nm was detected by an ELISA microplate reader (Zenyth, 3100) and the concentrations of IFN- γ , IL-12, IL-23, IL-1 β , and IL-17 levels in gingival fluids were determined. A standard curve was calculated using linear dilutions of the Reagent Diluent supplied by the manufacturer (29).

Statistics

Data are expressed as the mean $\pm$ SEM for each group. We tested for normality using the Shapiro-Wilk's test and for homogeneity of variances using Levine's test. A paired-samples t-test was used to compare the two matched groups. The independent samples Student's t-test was otherwise used to compare two groups with a Gaussian distribution. Statistical analyses were performed using SPSS 25.0 for Windows (SPSS, Inc., Chicago, IL). All reported p values were 2-sided, $p < 0.05$ was considered statistically significant, and $p < 0.001$ was considered highly significant.

RESULTS

Third of the UC patients were diagnosed with Lingua plicata

During a dental examination, 7 out of 20 UC patients (35%) (Fig 1B) were diagnosed with Lingua plicata. An increased number of fissures and grooves at the central and lateral aspects of the dorsal surface of the tongue were observed in UC patients (Fig 1A). None of these patients were aware of this malformation before the examination. Healthy patients were not diagnosed with LP.

The concentration of proinflammatory cytokines in GCF is significantly higher in UC patients compared to healthy subjects

It is well known that proinflammatory mediators have an important role in the progression of UC. We measured the levels of proinflammatory cytokines (IFN- γ , IL-12, IL-1 β , IL-23, and IL-17) in GSF and found that concentration of IFN- γ , IL-12, and IL-1 β was significantly increased in the group of UC patients when compared with healthy controls ($p < 0.05$, $p < 0.01$, $p < 0.001$; Figure 2A-C). Additionally, there was a significant difference in Th17 cytokines (IL-23 and IL-17) in GSF between UC patients and healthy controls ($p < 0.01$, $p < 0.05$; Figure 2D, E).

Higher concentrations of IFN- γ and IL-12 were measured in GSF samples of UC patients with LP

To analyze the possible association between inflammation in the oral cavity of UC patients and the development of LP, UC patients were divided into two groups according to the presence or absence of LP. Representative images illustrating UC patient with lingua plicata is shown in Figure 1A.

During a dental examination, out of 20 UC patients, 7 (35%) of UC patients were diagnosed with lingua plicata (Figure 1B). None of the patients were aware of this malformation before the examination. None of the healthy patients enrolled in this study were diagnosed with LP. The GCF levels of IFN- γ and IL-12 were higher in UC patients with LP compared with UC patients without LP ($p < 0.05$, $p < 0.05$; Figure 2F, G). There were no significant differences in GCF levels of other cytokines between UC patients with and without LP (data not shown).



Figure 1. UC patients with LP.

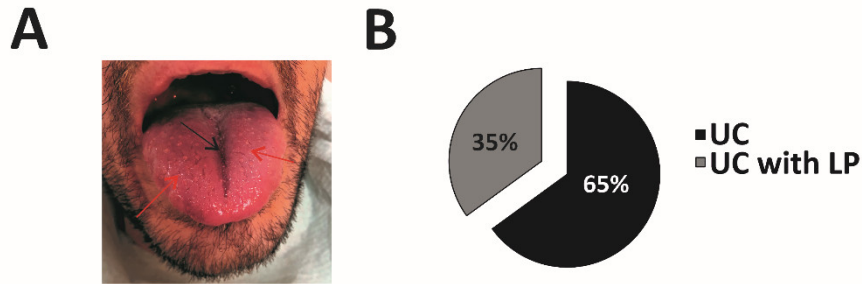
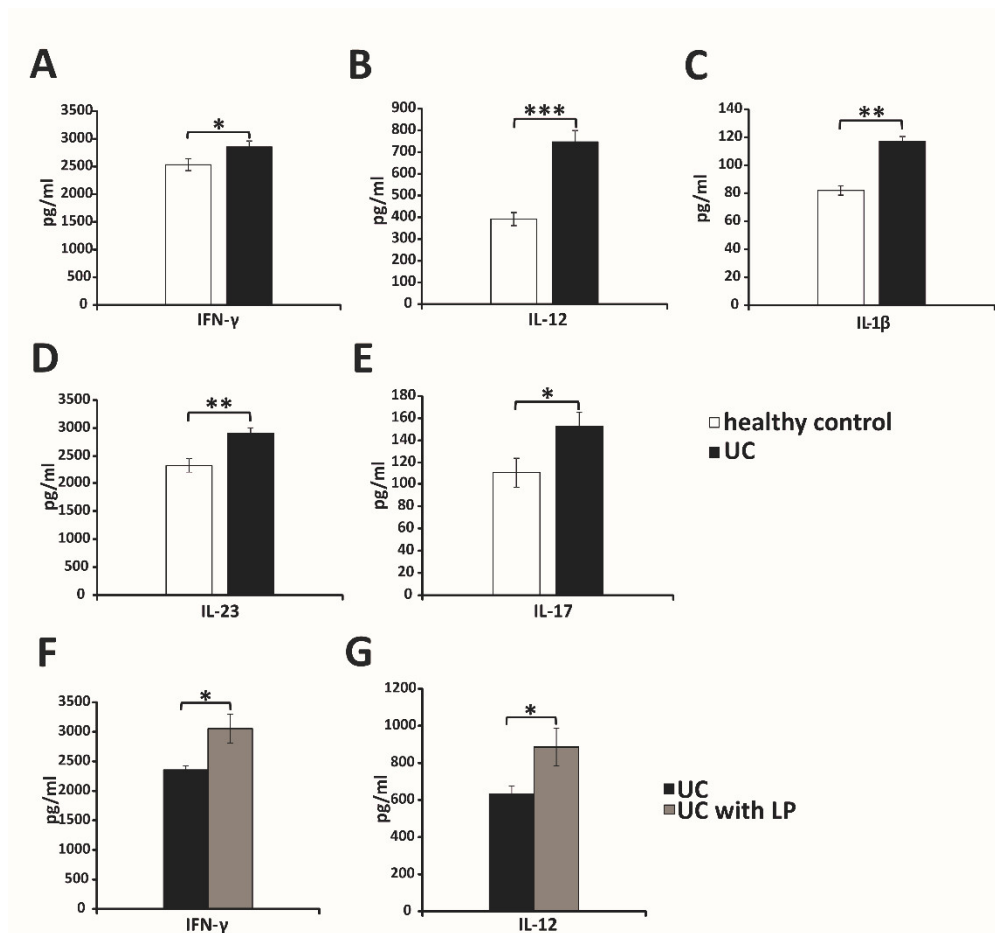


Figure 2. Cytokine profile of UC patients in GCF. (A-C)



Level of proinflammatory IFN- γ , IL-12, and IL-1 β in GCF. **(D, E)** A significantly higher concentration of Th17 cytokines (IL-23 and IL-17) in GCF.

Data presented as mean $\pm$ SEM; * p <0.05, ** p <0.01, *** p <0.001.

(F, G) Levels of proinflammatory IFN- γ and IL-12 in GCF obtained from UC patients diagnosed with LP. Values are mean $\pm$ SEM; * p <0.05.



DISCUSSIONS

In the present study, we compared the level of proinflammatory cytokines in GCF of UC patients and healthy individuals and we analyzed the association between GCF levels of inflammatory cytokines and the development of LP. Measurement of inflammatory cytokines in GCF may be used for the evaluation of the progression of systemic inflammatory diseases (1). We collected GCF from sites without any pathological signs. Our results have shown that UC patients have higher concentrations of proinflammatory cytokines (Fig 2A-E) compared to healthy controls. These results are in line with previously published data which demonstrated increased serum levels of these cytokines in UC patients (30), indicating the possible use of GCF in the monitoring of UC progression (30).

LP is a condition characterized by an increased number of fissures and grooves at the central and lateral aspects of the dorsal surface of the tongue (17). As all UC patients were *de novo* diagnosed with LP, we could not elucidate the influence of LP on UC severity. Our findings suggest that there is an association between a higher concentration of inflammatory cytokines in GCF and LP (Figure 1). Many studies (28-31) confirmed that increased concentration of proinflammatory cytokines in the saliva of IBD patients could be a direct connection to disease severity. Elevated levels of IL-6, IL-1 β , and TNF- α in the saliva were detected in patients with active Chron's disease along with the manifestation of the specific oral lesions (29, 30). Moreover, levels of proinflammatory cytokines are found to be increased in the saliva of patients with different oral pathologies: periodontal disease, lichen planus, and oral carcinoma (30, 31). In line with these results, we have been detected increased concentration of proinflammatory IFN- γ and IL-12 in GSF in patients with LP (Figure 2F, G).

Cytokines, such as IFN- γ and IL-12, play a pivotal role in the modulation of the intestinal immune system. They are considered to be major drivers of this excessive immune response, leading to massive leukocyte infiltration and mucosal damage (18, 32). Moreover, the expression of IL-12 is up-regulated in both active form UC and CD biopsies and it correlates with activity index score (33). Since IFN- γ and IL-12-producing immune cells of the oral cavity contribute to the development of systemic inflammation in UC patients, quantification of IFN- γ and IL-12 in GCF may provide useful information for the diagnosis of disease status.

In summing up, the analysis of GCF levels of inflammatory cytokines should be used for the monitoring of UC progression. Increased GCF values of IFN- γ and IL-12 in UC patients with LP may be considered as a sign of the disease aggravation and, consequently, of a poor prognosis for patients.

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