

Case Report

Chronic gingivitis and aphthous stomatitis relationship hypothesis: A neuroimmunobiological approach

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ABSTRACT

Background: Traumatic injuries to the oral mucosa in fixed orthodontic patients are common, especially in the first week of bracket placement, and occasionally lead to the development of aphthous stomatitis or ulcers. Nevertheless, these lesions are self-limiting. **Purpose:** The objective of this study is to reveal the connection between chronic gingivitis and aphthous stomatitis which is still unclear. **Case:** A patient with a persistent lesion for more than six months. **Case Management:** RAS was treated with scaling procedure, the gingival inflammation was healed. However, in this case report, despite the appropriate management procedures had been done, the lesion still worsen and became more painful. Moreover, the symptoms did not heal for more than two weeks. Actually, they had been undergone orthodontic treatment more than six months and rarely suffered from aphthous stomatitis. Coincidentally, at that time they also suffered from chronic gingivitis. It was interesting that after scaling procedures, the ulcer subsides in two days. **Conclusion:** Recently, the neuroimmunobiological researches which involved neurotransmitters and cytokines on cell-nerve signaling, and heat shock proteins in gingivitis and stomatitis are in progress. Nevertheless, they were done separately, thus do not explain the interrelationship. This proposed new concept which based on an integrated neuroimmunobiological approach could explain the benefit of periodontal treatment, especially scaling procedures, for avoiding prolonged painful episodes and unnecessary medications in aphthous stomatitis. However, for widely acceptance of the chronic gingivitis and aphthous stomatitis relationship, further clinical and laboratory study should be done. Regarding to the relatively fast healing after scaling procedures in this case report; it was concluded that the connection between chronic gingivitis and aphthous stomatitis is possible.

Key words: chronic gingivitis, aphthous stomatitis, orthodontic treatment, neuroimmunobiological approach

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INTRODUCTION

Hipocrates (460-370 AD) was the one who, for the first time, used the word “aphtha” (aphtodea), came from the Greek word *aptein* (ARTEIV), translated as “set fire”. Until today, the etiology of recurrent aphthous stomatitis (RAS) remains unknown, although hints of its etiological basis lay on genetic susceptibility, infectious agents and alterations in immune mechanics.¹ Others are deficiencies of iron, vitamin B, or folate had been reported in patients with RAS, but the data are conflicting.²

Infection with various microorganisms had been suggested but not proven to be a contributing factor, although cross-reactivity between a microbial antigen

and a homologous peptide (the heat shock protein theory) within the oral epithelium may play a role.² Various factors have been suggested to precipitate outbreaks of RAS in predisposed persons, including oral trauma, anxiety or stress, sensitivities to food (e.g., to preservatives), and hormonal changes related to the menstrual cycle. Nevertheless, evidence to support the causative role of these factors is rarely.³

In addition, RAS management does not change radically throughout time. Recent management protocols according to Scully in 2008 are still conventional medication such as topical corticosteroids, topical anti-inflammatory, topical antibiotics, and antiseptic mouthwash. If they failed, immunomodulators such as Thalidomide could be used.⁴

Because of the uncertain etiology of RAS, it may lead to inappropriate medication, a condition that may provide adverse effects after prolonged use; thus, other concept which related to oral homeostasis should be considered.

It is also a common sense that in fixed orthodontic treatment, brackets and wires are to be blamed as a prominent causative factor of RAS. Nevertheless, the role of fixed orthodontic accessories (i.e. brackets, modules, chains) as bacterial adherence media which may provoke RAS is often overlooked.^{5,6} We report two cases in which, RAS was found in orthodontic patients who rarely experienced the disease before. At that time they also suffered from chronic gingivitis adjacent to the lesion. Even though maintaining good oral hygiene is suggested in RAS management, scaling procedure is not mentioned specifically in RAS management protocol. In our cases the procedure resulted quicker healing compared to previous conventional treatment. However, the mechanism of the prompt healing of RAS symptoms after scaling procedure is still unclear.

Current literatures discussed about the role of cytokines, neuropeptides and heat shock proteins (HSPs) which have an important role in health and disease. The balance of these substances could be modified after exposure to stressors such as physiological (i.e. hormones), physical (i.e. trauma), pathophysiological (i.e. bacterial infection) and psychogenic.⁷ Additionally, oral inflammation such as chronic gingivitis is able to stimulate interactions of immunogenic and neurogenic inflammation, so called the “neurogenic switching” mechanism.⁸ Periodontal treatments reduce either local or systemic inflammation; i.e. by decreasing pro-inflammatory cytokines, neuropeptides^{8,9} and HSPs; furthermore, they also increase anti-inflammatory mediators i.e. HSP10.¹⁰

Pro-inflammatory HSPs 60 and 65 are abundantly found in chronic periodontal disease.¹⁰ Interestingly, RAS is also related with increase response to these HSPs.^{2,11} However, until now, the relationship between chronic gingivitis and RAS is still unclear. Moreover, even though scaling is regarded as a simple and routine procedure, the relationship with the etiopathogenesis and the healing process of the RAS lesion should be elucidated. Hopefully, after further researches, scaling procedure could be explicitly included in RAS management protocol.

The objective of this case report is to discuss evidence-based cases which may explain a new hypothesis concerning the possible relationship between chronic gingivitis and RAS. Successful researches verifying this hypothesis may include scaling procedure in RAS management protocol, therefore preventing from unnecessary medications. For this reason, since interaction of the neural, immune and biological system of the oral cavity is possible; this hypothesis will be elucidated through a neuroimmunobiological approach.

CASE

Case 1: A 14-year-old male orthodontic patient suffered from recurrent ulceration on the lower labial oral mucosa. The orthodontic treatment began one and a half year before the ulcer occurred, when the teeth had already aligned. Actually, he seldom complained about traumatic ulcer, if existed, it healed spontaneously. Nevertheless, despite antiseptic mouthwash and topical corticosteroid treatment, within the last one month the ulceration persisted for more than 2 weeks. They were multiple ulcers on the lower labial mucosa, about 3–4 mm in diameter, and painful.

Coincidentally, at that time he also missed several regular appointments, thus oral hygiene could not be controlled as usual. Intra oral examination revealed that the gingiva was bright red and severely inflamed, especially in the lower labial gingiva. At this region, the labial surface of the teeth were half covered with hyperthropic papillae and supragingival plaque. Moreover, the lingual aspects of lower anterior teeth were covered with calculus.

Case 2: A 13-year-old female orthodontic patient complained of recurrent ulceration on the oral mucosa. The orthodontic treatment was started a year before, she had dental protrusion without crowding. After brackets placement, ulcerations developed and usually healed within 1–2 weeks. Recently, ulcerations recurred and required more than 2 weeks to heal. Ulcerations developed on the various site on the non-keratinized mucosa. The last ulcer was found on the lower right labial mucosa, adjacent to the bracket placed on 42, round in shape, shallow, about 4 mm in diameter, grayish white, surrounded by erythematous halo, and painful. Self medication history for the oral ulceration included Chinese herbs and commercial mouthwash (Listerine®). The medication failed to promote healing. Intra oral examination revealed that supra gingival calculus was found on the lingual and labial sides of the lower gingiva, accompanied by inflammation on the marginal gingiva.

The diagnosis in these cases are RAS and Chronic Marginal Gingivitis. It is suggested that the gingival inflammation resulted from dental plaque and calculus is playing a role in the etiopathogenesis of both cases.

CASE MANAGEMENT

The male patient initially came for regular orthodontic control when the persistent ulceration was reported. The last visit was two months before. Since there was chronic gingivitis on the lower anterior gingiva, the first attempt was to reduce this lesion by scaling and not intentionally done for the RAS. RAS symptoms still treated by previous topical corticosteroid which actually was not very helpful in this case, and only acts for tissue protector. He was scheduled



Figure 1. Chronological intra oral photographs of aphthous stomatitis resolution.

for another visit one week later and was instructed to maintain good oral hygiene.

At the second visit, the result of scaling procedure was remarkable. The gingival inflammation was healed. Interestingly, the ulcer also subsided. Although it was still present but the diameter was smaller, only one mm, pink colored, and not painful anymore the lesion still present but only one mm in diameter and pink colored. The lesion did not elicit pain anymore. Additionally, his oral hygiene was good. Anamnesis revealed that the pain subsides significantly after two days, so did the lesion diameter. The patient was told to come to the dental office two weeks later for regular orthodontic control.

After experiencing similar cases in several RAS patients that responded well to scaling procedures, we became more convinced with this phenomenon. Therefore scaling procedure was also done as the treatment of RAS in the second case. Chronological pictures of the lesions were also documented. These figures were shown: pre-treatment lesion (Figure 1-a); 2 days after (Figure 1-b); 5 days after (Figure 1-c); one week after (Figure 1-d).

DISCUSSION

Chronic gingivitis is an inflammatory lesion of the soft tissues surrounding the tooth and a consequence of the local accumulation of dental plaque. It is a multifactorial disease, principally associated with infection by specific pathogenic organisms. Stimulation by bacteria, cell surface molecules

like lipopolysaccharides (LPS) and bacterial metabolites to immunocompetent cells are responsible for the initiation and early development of gingivitis. These immunocompetent cells release an array of pro-inflammatory mediators i.e. interleukin 6 (IL-6), prostaglandins.⁹

Recurrent aphthous stomatitis is a common condition, restricted to the mouth. In these patients the lesions were diagnosed as minor aphthous ulcers (2 to 8 mm in diameter) and usually heal spontaneously in 10 to 14 days.² However, the lesions were still painful and had insignificant healing after more than 2 weeks.

Even though the exact immunopathogenesis of RAS remains unclear, it probably involves cell-mediated mechanisms. Macrophages, mast cells and T cells probably aid in the destruction of oral epithelium that is directed and sustained by local cytokine release.^{2,3} Compared with control subjects, individuals with RAS have raised serum levels of cytokines such as IL-6 and IL-2R. Cross-reactivity between a streptococcal HSPs60 and 65, and the oral mucosa has been demonstrated, and significantly elevated levels of serum antibodies to HSPs are found in patients with RAS. It was predicted that RAS may be a T cell-mediated response to antigens of streptococcus i.e. *S. sanguis*, which cross-react with self-HSP and induce oral mucosal damage.^{3,11} Interestingly, the human HSPs 60 and 65 also implicated in the pathogenesis of periodontal disease and the involvement of T-cells response. Albeit RAS may be caused by the cross-reactivity between self-HSP and HSPs from periodontopathic bacteria i.e. *Porphyromonas*

gingivalis;¹⁰ nevertheless, this interactions scarcely discussed.

In chronic gingivitis, the inflammatory reaction is not purely immunogenic, since according Lundy and Linden, the role of nervous system, especially the primary afferent neurons should be taken into account. This neurogenic inflammation involved neurotransmitter, the neuropeptides. The pro-inflammatory neuropeptides are substance P (SP) and calcitonin gene-related peptide (CGRP); and the anti-inflammatory is vasoactive intestinal peptide (VIP).⁸ Studies have shown that most neuron expressing SP are nociceptors (related to pain), whereas those expressing CGRP may belong to either nociceptive or non nociceptive afferents.¹²

These neuropeptides increased expression in local nerve in the inflamed periodontium. In their animal study, Saleem *et al.*,¹² concluded that the up-regulated CGRP in periodontal disease may be principally associated with neuroimmune interaction rather than nociceptive spinal inputs. It is agreed by Wadachi and Hargreaves¹³ that chronic periodontal disease did not cause pain. Saleem, *et al.*,¹² study revealed that the increase of CGRP after stimulation in one branch of the trigeminal nerve also propagate to the other branch; increase of CGRP also stimulates VIP secretions to counteract.^{8, 14} Moreover, there is a special characteristic of this anti-inflammatory neuropeptide which acts as a long-lasting vasodilator.¹⁴ If VIP present persistently in the buccal or labial mucosa there should be a persistent mucosal inflammation that is prone to trauma from biting or brackets.

Several studies by Kempainen *et al.*,¹⁵ support this proposed mechanism. Kempainen *et al.*,¹⁶ showed that pulpal stimulation which considered as neurogenic inflammation was able to induce lip vasodilation, nevertheless, the exact mechanism was still unclear. Another study by Kempainen *et al.*,¹⁶ also revealed that non-keratinized mucosal tissue is prone to nerve stimulation which leads to neurogenic inflammation. Interestingly, either sulcular epithelium or labial mucosa are non-keratinized mucosa. Therefore pseudopockets in chronic gingivitis facilitate the initiation of the neurogenic inflammation

In inflammatory reaction, prostaglandin E₂ (PGE₂) increases the sensitivity of primary afferent nerve fibers to stimuli.¹⁷ Interestingly, that according to Burt epidemiological study, chronic gingivitis had the higher PGE₂ level in gingival crevicular fluid than periodontitis-only.¹⁸ Therefore, nerve stimulation in chronic gingivitis is considered easier than periodontitis-only or healthy periodontium.

Other literatures revealed that local inflammation could be propagated to distant organ via the nervous system, this mechanism often called as “neurogenic switching mechanism”.¹⁹ Moreover, according to Boyd, stimulation of the maxillary nerve in the nasal cavity also stimulates branches of the maxillary nerve in the temporal area via neurogenic inflammation.²⁰ Does it also happen in the mandibular nerve innervation area as in this case report?.

Periodontopathic bacteria and their products are able to stimulate immunocompetent cells which then initiate the neurogenic switching mechanism. Lipopolysaharides (LPS) from Gram negative bacteria is able to stimulate these cells which then release mediators to activate primary afferent nerve endings.⁸ This mechanism also verified in Saleem *et al.*,¹² study, since LPS injection in the mandibular gingiva also increase CGRP expression in the trigeminal ganglion, began with the mandibular followed by maxillary and ophthalmic branch.

Heat shock proteins (HSPs) are normally intracellular proteins that have functions involved in protein folding and maintenance of protein integrity under both normal and stress conditions, yet if they are to act signals in response to environmental stresses (i.e. infection, temperature, pH, redox potential, physiological stress), they should be present in extra cellular environment.⁷ Infection or other environmental stress stimulates self-HSPs including the self-HSP60 (pro-inflammatory HSP) to appear, it is beneficial for stimulating macrophages to produce pro-inflammatory cytokines in an inflammatory process. Unfortunately, HSP60 also act as lipopolysaccharides binding protein (LBP) which increase LPS immunogenicity, thus exaggerate the inflammatory process, which is an unwanted effect.²¹

Local and systemic HSPs are increase in periodontal disease. Yamazaki *et al.*,²² revealed that in chronic gingivitis, serum antibody to self-HSP60 was increased. In addition, according to Ueki *et al.*,²³ self-HSP60 expressed abundantly in periodontal lesion and similar to bacterial LPS, thus it is able to stimulate TNF- α production by macrophages. Periodontal treatment is able to decrease inflammation by increasing anti-inflammatory HSPs (i.e. HSP10) that inhibit pro-inflammatory HSPs.¹⁰ It also reduce pro-inflammatory cytokines; neuropeptides SP, CGRP and VIP.^{9,14}

It is common that initial bracket placement will cause trauma to buccal or mucosal tissue which may cause RAS. Nevertheless the frequency will reduce after adaptation. In orthodontic patients, orthodontic braces, wires and accessories made them more susceptible to food impaction and difficulties in maintaining optimal oral hygiene. Therefore, supragingival and subgingival plaque which also called biofilm accumulated. The developing pseudopockets in chronic gingivitis made the subgingival plaque elimination became more difficult. Subsequently, there were increase of infectious agents which also increase either self-HSP60 or bacterial-HSP60. This condition also facilitates activation of immunocompetent cells by LPS to generate the neurogenic switching mechanism.

Several literatures explained the cross-reactivity between microbes and oral epithelial cells, which are active components of the innate immunity. *Porphyromonas gingivalis* components: i.e. fimbriae, gingipains and LPS, are able to elicit gingival epithelial cells (GECs) reaction via molecular pathway. Other current finding revealed that *P. gingivalis* are able to invade epithelial cells (intra-epithelial) *in vivo* (Figure 2).^{24,25}

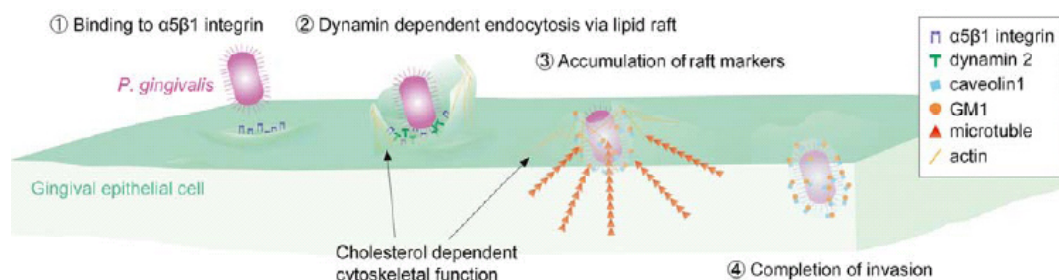


Figure 2. Epithelial internalizing by *Porphyromonas gingivalis*.²⁹

Moreover, *P. gingivalis* also able to degrade epithelial cell junctional complex and the infection can be transmitted cell to cell without passing through the extra cellular space.²⁵ This mechanism of spreading may allow *P. gingivalis* to colonize oral tissues without exposure to the humoral immune response.²⁶ It is also interesting that *P. gingivalis*, which commonly colonized in the subgingival plaque, a study by Suzuki *et al.*,²⁶ found their existence in saliva; and Hafajeel *et al.*,²⁷ in the supragingival plaque. These literatures support the possible involvement of periodontopathic bacteria such as *P. gingivalis* in RAS. *P. gingivalis* could act as intraepithelial bacteria, thus is more resistant to antiseptic mouthwash as in this case report.

Recent hypotheses postulate RAS are a consequence of an autoimmune reaction against oral epithelium. It has been suggested that this autoimmune reaction could be a cross-reaction immune response, activated by HSPs released by oral bacteria and targeting similar peptides in the oral epithelium. Therefore, in our opinion there should be a possible bacteria similarity between chronic gingivitis and RAS; nevertheless, it was contradictory with Marchini *et al.*,²⁸ study which found that among periodontopathic bacteria only *Prevotella* sp which appear in 16% RAS sufferers, *Porphyromonas* sp was not significantly detected in samples. However, this result was based on bacterial study of the buccal mucosa swab samples only, and did not investigate the intraepithelial bacteria. The role of intraepithelial bacteria such as *P. gingivalis* in RAS is supported by a recent clinical study; the application of topical minocycline which usually indicated for periodontal disease was successful for RAS treatment. Minocycline, besides its microbial effect, it also a host response modulator and able to give effect intraepithelially.²⁹

It is likely that elastomeric modules and chain act as media for plaque accumulation. Nevertheless, a study by Ahn *et al.*,⁵ showed that despite its “smooth” surface, bacteria also adhere to brackets, especially on metal.⁶ Therefore, optimal oral hygiene maintenance and regular changing of the elastomeric accessories should be conducted. These orthodontic patients wear metal brackets and elastomeric modules, with poor oral hygiene; thus more susceptible to bacterial adhesion and facilitate contacting bacterial colony to the buccal or lip mucosa.

Based on these literatures, the chronic gingivitis and RAS relationship hypothesis could be explained by neuroimmunobiological approach as follows: 1) PGE2 in chronic gingivitis lowered activation threshold of afferent primary nerve fibers which become more sensitive to stimuli; 2) activation of immunocompetent cells by LPS release mediators that able to activate nerve endings which then produced neuropeptides; especially in the same innervation area (in this case was the lower lip); 3) prolonged sensitization increase VIP level which not easily degraded that also lead to long lasting vasodilatation of tissue vessels, including the buccal or lip mucosa which become more inflamed; 4) the presence of intraepithelial bacteria and the cross-reactivity of HSPs exaggerate the ongoing mucosal inflammation. Therefore, the affected lip or buccal mucosa became more susceptible to trauma from brackets or unintentional biting. The disrupted epithelial junction by TNF- α worsen the effect.

For the concluding remarks, since the interesting phenomenon, RAS lesion which relief after scaling procedures could be explained by neuroimmunobiological perspectives, thus our hypothesis is possible. Moreover, this discussion could explain the uncertain etiopathogenesis of RAS, and in our opinion, the cause of RAS is rather locally, but could be exaggerated by systemic condition (i.e. stress, immunocompromise). Nevertheless, more evidence based cases and randomized controlled trials should be done and supported by immunopathological and microbiological examination to verify this hypothesis.

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