치의과학박사 학위논문

Salivary Levels of Cortisol, 17β-Estradiol, Progesterone, Dehydroepiandrosterone and α-Amylase in Patients with Burning Mouth Syndrome

구강작열감증후군 환자의 타액 Cortisol, 17β-Estradiol, Progesterone, Dehydroepiandrosterone, α-Amylase 연구

2013년 2월

서울대학교 대학원

치의과학과 구강내과 · 진단학 전공

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논문제목: Salivary Levels of Cortisol, 17β-Estradiol, Progesterone,

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ABSTRACT

Salivary Levels of Cortisol, 17β-Estradiol, Progesterone, Dehydroepiandrosterone and α-Amylase in Patients with Burning Mouth Syndrome

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Burning mouth syndrome (BMS) is characterized by a painful burning sensation or other dysesthesias of the oral mucosa, with no visible mucosal abnormalities upon clinical examination, so that seriously exacerbates quality of life. The anatomical proximity between saliva and the area of BMS symptoms and the importance of steroid hormones in the pathophysiology of BMS have resulted in the investigation of possible salivary biomarkers. The aim of this study was to investigate salivary cortisol, 17β-estradiol, progesterone, dehydroepiandrosterone (DHEA) and α-amylase levels in patients with BMS compared with controls and to investigate whether these levels could be predictors for treatment outcome in patients with BMS. Thirty female patients with BMS and twenty female control subjects were included. Unstimulated whole saliva (UWS) and stimulated whole saliva (SWS) samples were collected, and their flow rates were determined. Salivary levels of cortisol, 17β-estradiol, progesterone and DHEA were analyzed using enzyme immunoassay kits. The enzymatic activity of α-amylase was determined using maltotriose as a substrate. Salivary transferrin level was measured to determine the level of blood contamination in saliva samples. Symptom checklist-90-revision (SCL-90-R) was used for

psychological characteristics of patients with BMS. Treatment protocols of patients with BMS included control of parafunctional habits, use of artificial saliva, and clonazepam medication.

The obtained results were as follows:

1. The patient group showed significantly higher levels of cortisol in UWS ($P < 0.05$) and of 17β-estradiol in SWS ($P < 0.05$).

2. When the patients were divided into older (≥60 years) and younger (<60 years) groups, the older group showed a significantly lower level of progesterone in UWS ($P < 0.05$).

3. There was no significant correlation between all scales of SCL-90-R and the levels of salivary analytes.

4. There was no significant correlation between the treatment efficacy and the levels of salivary analytes.

In conclusion, patients with BMS had significantly higher levels of cortisol in UWS and of 17β-estradiol in SWS. These indicate that dysregulations of the hypothalamic-pituitary-adrenal (HPA) axis and gonadal steroids are involved in the pathogenesis of BMS.

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I. INTRODUCTION

Burning mouth syndrome (BMS) is characterized by a painful burning sensation or other dysesthesias of the oral mucosa, with no visible mucosal abnormalities upon clinical examination. Multiple sites in the oral cavity are usually affected with the tongue being the most common area.[1,2] BMS usually affects aged women; about 90% of women who attend healthcare clinics for BMS symptoms are peri/postmenopausal.[3] The etiology of BMS is multifactorial, probably involving complex interactions among local, systemic, and psychogenic factors.[4] Based on the recent findings,[5-7] BMS is believed to be a neuropathic pain entity involving the peripheral and/or central nervous system.

It has been suggested that altered levels of female sex hormones in the peri/postmenopausal period may predispose women to BMS.[3,8] Disturbances of local neuroactive steroids have also been suggested to explain the very restricted location of the burning sensation.[7] In addition, previous studies found that chronic stress and/or adverse life events of long duration correlate with BMS, suggesting an association of BMS with dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic nervous system.[7,9-12] Therefore, comprehensive information about changes in female sex hormones, local neuroactive steroids, and molecules related with dysregulation of the HPA axis and sympathetic nervous system is needed.

Like other chronic pain disorders, BMS is characterized by accompanying psychological problems. Personality and mood changes, especially anxiety and depression, are consistently demonstrated in patients with BMS, leading to the suggestion that BMS is a psychogenic problem.[13] BMS has also been suggested as a complex somatoform disorder because of the high prevalence of unexplained extraoral comorbidities.[14]

The treatment for BMS is usually focused on symptom relief and is similar to the medical management of other neuropathic pain conditions.[2] Among pharmacological options including benzodiazepines, tricyclic antidepressants, anticonvulsants, capsaicin, and α-lipoic acid,[4] clonazepam therapy is the most widely accepted.[15-18] However, clonazepam therapy is not always effective for all patients with BMS because of the

complexity of BMS pathogenesis.[5,19,20] In addition to drugs, psychological interventions can sometimes help patients cope with the symptoms of BMS.[21]

Recent advances in the etiology, pathophysiology, and management of BMS have lead to an interest in finding diagnostic and prognostic BMS biomarkers.[8-11] The anatomical proximity between saliva and the area of BMS symptoms and the importance of steroid hormones in the pathophysiology of BMS[7] have resulted in the investigation of possible salivary biomarkers. Moreover, the use of saliva for diagnostic purposes has received special attention, as saliva is easily obtained non-invasively, repetitively, and by individuals with limited training.[22] The levels of salivary steroid hormones are of clinical importance because of their excellent correlations with free serum levels.[22,23] In addition, salivary α-amylase has been proposed as a biomarker reflecting the sympathetic nervous system activity.[24,25]

The aim of this study was therefore to investigate salivary cortisol, 17β-estradiol, progesterone, dehydroepiandrosterone (DHEA), and α-amylase levels in patients with BMS compared with controls and to investigate whether these levels could be predictors for treatment outcome in patients with BMS.

II. REVIEW OF LITERATURE

1. General aspects of BMS

(1) Definition and epidemiology

BMS is a burning sensation in the oral cavity with clinically normal oral mucosa, in which no medical and dental cause is existed.[2] The tongue is the primary location of the burning complaint in the majority of cases.[1] This principle clinical feature of oral mucosal pain is often accompanied by symptoms of dysgeusia and xerostomia, with or without the presence of salivary hypofunction.[3,23,27]

The prevalence of BMS has been reported to range from 3.7% to 18% (or even up to

40%) in older age groups,[1] especially in postmenopausal women.[2,28] This wide range of prevalence of BMS is obviously due to rather loose diagnostic criteria applied in earlier studies that has resulted in heterogeneous patient populations in many studies performed before the more distinct definitions of primary BMS and secondary BMS were launched.[1,3]

(2) Etiology and pathophysiology

The etiopathogenesis of BMS is still unclear. The obvious fact is that BMS represents a complex of multiple diseases with overlapping symptoms. Several evidences reveal local / systemic factors in the majority of patients suffering from BMS symptoms.[29-33] This subgroup of patients is classified as "secondary" BMS due to local / systemic factors, removal or treatment of these contributing factors resulting in clinical improvement. In this context, therapeutic failures might be explained by an underlying irreversible neuropathic damage or disorder which can result in the persistence of BMS even after removal of precipitating factors.[34] The remains of BMS patients are classified as "primary" BMS in whom it is not possible to identify clear etiological factors and who are, therefore, particularly difficult to manage and treatment.[3]

1) Local factors

As far as local factors are concerned, there is strong evidence for local nerve trauma, oral parafunctional habits, and salivary gland dysfunction.

The observation of taste changes and / or dysfunctions in BMS has suggested that a peripheral nerve injury is associated with BMS because oral burning sensation exhibits similar patterns observed in some inflammatory neural conditions (neuritis) or regional nerve trauma (neuroma).[35] In addition, some patients with taste dysfuction show a loss of inhibitory interactions between the central projection areas of the chorda tympani or glossopharyngeal taste nerves following peripheral injury to either nerve.[36,37]

Several studies report that parafunctional habits are observed in patients with BMS.[38,39] Parafunctional activity appears to be influenced by various exogenous factors, such as stressful life events, alcohol abuse, some personality characteristics, and psychiatric

or neurological pathologies.[40] The parafunction (especially night bruxism) is probably the result of an interaction between the limbic system and the motor system, but the dopaminergic system might also be involved.[41-43]

Decrease in salivary flow rate by radiation therapy, some systemic diseases, and various pharmacologic agents might play a role in the onset of this syndrome.[44-46] Salivary dysfunction have reportedly been associated with increased incidence of BMS.[47,48] It has been suggested that, in some cases, BMS results from either a reduction in salivary output (volume)[38,49,50] or a decrease in the salivary components (glycoproteins) required for lubricating and protecting the oral mucosa.[48,51]

2) Systemic factors

Several systemic factors may influence the prevalence, development, and severity of BMS symptoms. The most significant systemic predisposing conditions for BMS are menopausal disorders, diabetes, and nutritional deficiencies.

Approximately 90% of women who attend healthcare clinics for their BMS symptoms are peri/postmenopausal women.[3] In an attempt to understand a possible explanation for this association, researchers examined several features of menopause in BMS women. The duration and the type (e.g., natural, surgical, etc.) of menopause as well as the treatment-related features do not appear to play a pivotal role in either BMS development or severity.[49] The most possible theory regards menopausal hormonal changes as a "master player" in BMS onset,[52] although estrogen replacement therapy (ERT) does not relieve pain in many cases.[53,54] The variable response to ERT treatment may be due to either the presence/absence of the expression of nuclear estrogen receptors in oral mucosa[52] or the possible activation of reversible/irreversible neuropathic mechanism(s).

The association between BMS and nutritional deficiencies has also been examined. Occasionally, BMS patients exhibit low levels of blood serum vitamins B_1, B_2, and B_6,[55,56] but a decrease in serum vitamin B_{12}[57] is the most common finding in this subgroup of patients. Vitamin B complex replacement therapy, however, often proves ineffective for pain relief.[55,56] Other minor findings of nutritional deficiency in BMS subjects may include

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low levels of blood serum folic acid and iron,[56] suggesting a possible role of some type of anemia in the pathogenesis of this syndrome.[3]

The correlation between diabetes mellitus and BMS is still controversial. It has been suggested that type II diabetes mellitus plays a role in BMS development.[38,58] In contrast, other studies report a lack of association between these two conditions.[59-61] A possible explanation for this controversy may be that these diabetic patients were erroneously classified as BMS. In fact, at the time of the above studies, a lack of strict criteria for BMS diagnosis could have affected the selection of the patients. For instance, burning oral complaints in diabetic subjects, who are more prone to oral infections, are probably caused by oral candidiasis.[62] However, the lack of data cannot exclude the possibility that the alteration of pain thresholds in this BMS subgroup is related to the neuropathy,[63] which is a common complication in type II diabetes mellitus.

3) Psychological factors

Many BMS patients exhibit high levels of anxiety and depression as well as pain relief after suitable administrations of psychotropic drugs/medications such as anti-depressants or benzodiazepines.[3] However, there is increasing controversy as to whether depression and anxiety are primary[64] or secondary events[51] to the oral pain. It is noteworthy that psychological dysfunctions are common within patients with different types of chronic pain conditions. No association has been found between BMS development and stressful life events, even in the cases with high levels of psychological distress.[27] In addition, depression, anxiety, and somatic complaints subsequent to emotional/psychosocial stresses may be absent in BMS patients, and there may be only a few disruptions in their normal activities due to oral burning.[65] Finally, BMS patients with psychological disorders frequently show other precipitating factors, such as masticatory muscular tensions, denture design errors, and parafunctional habits, all of which are strictly associated with anxiety and depression in these individuals.[39,66] These findings do not seem to support the hypothesis that BMS is primarily a psychogenic disorder. On the contrary, they draw interest to an extensive psychogenic part of the pain in symptoms of BMS,[67] which may

result from the patients' difficulty in coping with their suffering and/or emotional distress.[68]

 4) Neuropathic influences on BMS

Taste changes and/or sensory dysfunctions have been observed in many BMS patients, suggesting neuropathic alterations in BMS.[51,69] In the last decade, the neuropathic origin of BMS has been emphasized by findings suggesting that there is an underlying disorder of the autonomic innervations of the oral cavity in BMS patients. It has been documented, in fact, that BMS patients may show: (1) abnormal perception of intensities in the pre-pain range and disturbances in the perception of non-nociceptive and nociceptive thermal stimuli,[70] (2) raised trigeminal nerve sensitivity and alterations in neuronal transmission,[71] and (3) disturbances of the mucosal neurovascular microcirculatory system.[72] These findings suggest peripheral alterations in the function of the sensory trigeminal nervous system in BMS. In further support of these preliminary results, it should be noted that electrophysiological examination reveals an abnormal blink reflex (BR) in BMS subjects.[73] This reflex is under dopaminergic inhibitory control through the basal ganglia connection with the facial motor nuclei,[74] and an abnormal blink reflex is also a common finding in extra-pyramidal disorders such as Parkinson's disease and facial dyskinesias.[75,76] In these conditions, the abnormal reflex is thought to be due to a deficient dopaminergic striatal influence on the brainstem nuclei.[77] These considerations, together with the very recent evidence of a decreased dopaminergic inhibition in BMS subjects by Fluordopa-Pet scans,[74] lead one to suggest that BMS is a disorder of the nigrostriatal dopaminergic system, which would primarily affect the regulation of nociception of the trigeminal system, and thus cause a loss of sensory inhibition.

A more recent study provides further support for the hypothesis that a neuropathic dysfunction is involved in BMS.[5] These investigators used quantitative sensory testing (QST) in addition to the BR recordings in a large group of BMS patients. This study is very important, because it is the first attempt to evaluate the peripheral and central neural pathways of the trigeminal system in a large group of BMS patients. There was considerable heterogeneity in the findings, with some patients showing signs of large-fiber

neuropathy, others of small-fiber neuropathy, and about one-fifth of the patients showing signs of increased excitability of the trigeminal system. In most patients, however, a link between the electrophysiological signs of sensory disturbance and an anatomical alteration was not possible and, furthermore, was not strictly confined to the site of the pain. Overall, the authors interpret their findings as suggestive of a generalized, possibly multilevel abnormality in the processing of somatosensory information in BMS, with electrophysiological evidence pointing to a peripheral neurogenic mechanism in the majority of patients.

2. Biomarker research of BMS at present

(1) Biomarker candidates for BMS research

  1) Cytokines

Cytokines are closely associated with central neurotransmitters and cytokine regulation is affected by stress. A number of studies have investigated a possible role for cytokines in major psychiatric disorders[78] and it has been implicated in the development of BMS.[79-81] IL-2 is an immune regulator playing a major role in inflammatory reactions as well as in tumor control. During inflammation, IL-2 stimulates secretion of proinflammatory cytokines such as IL-1, TNF-α and TNF-β.[82] IL-6 is a pleotropic cytokine that influences the antigen-specific immune responses and inflammatory reactions. Together with IL-1 and TNF-α (which also stimulate IL-6 secretion), IL-6 belongs to the group of main proinflammatory cytokines.[83]

  2) Serotonin

The neurotransmitter serotonin (5-HT) plays an important role in the configuration of mood, emotion, cognition and motor functions. This monoamine is synthesized from its precursor tryptophan and metabolized to 5-hydroxyindoleacetic acid.[84] Several findings on platelet/blood serotonin levels have been reported in various psychiatric, neurological, and immune system disorders.[85,86]

3) Immune proteins

Soluble forms of innate immune associated proteins including CD14 and toll-like receptor-2 (TLR-2) have been reported in adult human saliva.[87,88] CD14 and TLR-2 belong to a family of proteins called the pattern recognition receptors (PRR). The PRRs recognize conserved microbe/pathogen associated molecular patterns (M/PAMP) typically shared by a large group of microorganisms.[89] CD14 is widely considered as a co-receptor for TLR-4 mediated recognition of lipopolysaccharides of Gram negative bacteria.[90] TLR-2 interacts with multiple M/PAMPs including peptidoglycans of the Gram positive bacterial and the lipopeptides of mycobacterial cell wall.[91,92] CD14 has also been shown to associate with TLR-2 in recognizing specific MAMPS such as LPS of *Porphyromonas gingivalis* and a leucine-rich protein of *Bacteroides forsythus*.[93,94] Oral epithelial cells stimulated via TLR-2 and CD14 respond by secreting inflammatory cytokines such as IL-8.[87,88] Identification of soluble forms of some TLRs is suggestive of additional levels of regulation in the processes of host microbial recognition and immune activation.[88,95]

4) Salivary proteins

The submandibular gland functions as an endocrine gland secreting several compounds such as glycosaminoglycans (GAG) [hyaluronic acid (HA) and chondroitin sulfate (CS)], several proteins and enzymes into the saliva as the glandular kallikrein.[96] Active kallikrein cleaves the high molecular weight kininogen (HK), resulting in a burst of active kinins release in the proximity of its receptors. These peptides are short-lived hormones that serve as key regulators of local blood pressure and inflammatory response by increasing intracellular Ca^{2+} levels, stimulating nitric oxide production, and enhancing prostaglandin biosynthesis.

5) Neuropeptides

It is becoming increasingly evident that periodontitis and indeed other orofacial inflammatory disorders maybe modulated by imbalances in certain neuropeptides. In

periodontitis affected subjects, levels of both substance P (SP) and neurokinin A (NKA) were significantly elevated in gingival crevicular fluid of disease-affected teeth compared with healthy sites. The role of neuropeptides in exerting a trophic effect on peripheral tissues is also worthy of mention. Such system has been proposed to act tonically so that normal sensory stimuli would produce a continuous outflow of sensory transmitters whose actions would maintain tissue integrity.[97]

It is also important to note that nociceptive signals from periphery may be amplified or diminished during spinal processing, resulting in either central sensitization or desensitization, respectively. The stimulation of capsaicin-sensitive fibers [SP, calcitonin gene-related peptide (CGRP)] induce, on the one hand, an orthodromic noxious-sensory signal travelling toward cortical areas, and on the other hand, anaxoaxon reflex of primary sensory fibers which antidromically releases transmitters from peripheral terminals evoking a number of changes in peripheral substrates (neurogenic inflammation, gland secretion, smooth muscle contraction). Therefore, it has been claimed that capsaicin-sensitive neurotransmitters are implicated in the pathophysiology of local reactions accompanying painful conditions. Despite the presence of degrading enzymes, saliva appears to be fluid which seemingly being close enough to peripheral receptors for sensory neuropeptides, can offer an image of the peptides release by sensory endings, roughly mirroring the central release pattern. In fact, capsaicin-sensitive neuropeptides (SP, CGRP) have unique property in that impulses may travel orthodromically or antidromically: therefore the transmitter may be released in both central and peripheral neural endings.[98]

A number of neuropeptides exert a biologic effect that may play a role in the clinical presentation of BMS. SP, NKA and CGRP for example all have active vasodilatory effects and the latter two play a direct role in pain. Furthermore they have attracted a considerable amount of attention in other pain syndromes including fibromyalgia and temporomandibular disorders.[99]

Nerve growth factor (NGF), the prototypical member of neurotrophin family, is crucial for survival of nociceptive neurons during development and has been shown to play an important role in nociceptive function in adults.[100-102] In addition to neuronal sensitization,

NGF directly interacts with some immune cell types. Mast cells (MC) are considered important components in the action of neuropeptides.[103] There is strong evidence for functional interactions between MC and nerves in human oral mucosa.[104] MC might play an important role in BMS tissue since, upon stimulation by neuropeptides, they release a number of mediators involved in inflammation and neuropathic pain (e.g. tryptase) as well as NGF itself.[103,105-109] Furthermore, NGF and NGF-induced MC activation can lead to neutrophil accumulation which maybe critical for the sensitizing actions of NGF,[110,111] but may also have an anti-inflammatory and anti-nociceptive role via calprotectin, capable of inhibiting inflammatory pain in mice.[112]

6) Trace elements

It was reported that serum copper (Cu) concentration in depressive patients was significantly higher (by 21%) compared with that in controls.[113] Some studies suggested that magnesium (Mg) deficit occurs in patients with anxiety, depression and psychological complaints.[114,115] Other study suggested that lower serum zinc (Zn) in depression is in part explained by lowered serum albumin and by another depression related mechanism.[116] It is suggested that lower serum Zn in depression may be secondary to sequestration of metallothionein in the liver, which may be related to increased production of IL-6.

(2) Blood biomarker research for BMS

Xia et al.[81] reported that there was no significant difference in serum IL-2 and IL-6 between BMS patients with depression and without depression. Vucićević-Boras et al.[117] concluded that evaluation of tumor markers in patients with BMS was useless and in terms of cost-benefit the evaluation should not be performed in BMS patients. Chen et al.[118] showed that serum IL-6 in BMS patients was decreased and negatively correlated to pain level. Pekiner et al.[119] reported that serum IL-2 and TNF-α levels were significantly decreased in BMS patients compared with controls. Loeb et al.[120] revealed that serum serotonin level was decreased in BMS patients. Guimaraes et al.[121] showed that IL-1β high production genotype was increased in BMS patients.

(3) Salivary biomarker research for BMS

Simcic et al.[80] showed that salivary IL-2 and IL-6 was increased and correlated to pain severity in BMS patients. Vucićević-Boras et al.[79] concluded that there was no difference in salivary IL-6 and TNF-α between BMS patients and controls. Loeb et al.[120] reported that chondroitin sulfate concentration was decreased and kallikrein activity was increased in saliva of BMS patients. Srinvasan et al.[122] displayed that the level of soluble TLR-2 (sTLR-2) was upregulated in saliva of BMS patients. Pekiner et al.[123] exhibited that the level of Mg was decreased in saliva of BMS patients, although there was no difference in the level of Zn, Cu, IL-2 and IL-6 between BMS patients and controls.

Zidverc-Traikovic et al.[124] expressed that the level of CGRP was no difference in BMS patients in comparison to healthy subjects. Suh et al.[125] showed that there was no difference in the salivary levels of IL-1β, IL-6, IL-8, and TNF-α between BMS patients and controls. Borelli et al.[126] reported that NGF and tryptase activity was increased in saliva of BMS patients although the level of SP was decreased in saliva of BMS patients.

3. Salivary biomarker research for BMS in the future

(1) Importance of saliva as a diagnostic tool

Saliva offers distinctive advantages over serum because it can be collected non-invasively, repetitively, and by individuals with limited training. The correlation between saliva and plasma levels make saliva a valuable clinical tool. Furthermore, micromethodological and molecular biological advances over the past decades have enabled saliva to expand its usefulness in the diagnosis of disease, prediction of disease progression, monitoring of therapeutic drug levels and detection of illicit drugs.[127-129]

(2) The dysregulation of sympathetic nerve and hypothalamic-pituitary-adrenal (HPA) axis in BMS

Pain sensation, and modulation and control of autonomic output are integrated by a

central network that is critical for adaptation and survival. There are extensive interactions between the nociceptive and the autonomic systems at the levels of the central and peripheral nervous systems. Especially, it is well known that the change of sympathetic nerve function and HPA axis activity is closely related to chronic pain as headache, temporomandibular disorders and fibromyalgia.[130] The fuction of sympathetic nerve system represents as the activity of epinephrine / norepinephrine and the activity of HPA axis represents as the level of serum cortisol concentration.

It has been suggested that salivary α-amylase activity reflect the activity of sympathetic nerve system.[131] The concentration of salivary cortisol is closely correlated with the concentration of serum cortisol and salivary cortisol appears to be a valid measure for HPA axis activity.[132]

1) Relationship of sympathetic nerve system with BMS

Among the pathophysiology of BMS, it was reported that BMS was caused by degenerations of peripheral nerves and the functional change of central nerve system.[133] It seems that BMS is relative to the dysregulation of sympathetic nerve system likewise other chronic pain disorders. Accordingly, the further evaluation is mandatory about the possibility that the dysregulation of sympathetic nerve system represents as prognostic biomarker of BMS.

2) Relationship of HPA axis with BMS

Several studies revealed that high level of anxiety and depression correlated with BMS and chronic stress with long duration was related to BMS, suggesting that symptom and severity of BMS is related with the dysregulation of HPA axis.[10,11] Accordingly, the further study is necessary about the possibility that the dysregulation of HPA axis represents as prognostic biomarker of BMS.

(3) Dysregulation of sex steroid hormone in BMS

It is well known that the levels of salivary steroid hormones are closely correlated with

free serum levels.[127,128] The fact that chronic pain disorders generally affect women is applied to chronic pain disorders of orofacial region. Almost all patients with BMS are peri/postmenopausal women. It has been suggested that altered levels of female sex hormones in the peri/postmenopausal period may predispose women to BMS.[3,8] Accordingly, the further examination is essential about the relationship of the altered level of salivary sex hormone with the severity and intensity of BMS.

(4) Dysregulation of neuroactive steroid in BMS

The fact that the burning sensation is retricted to oral cavity is suggested that disturbances of local neuroactive steroids also play an important role in BMS. Neuroactive steroids may explain the restricted location of the symptoms: they are synthesized by nearby cells and are active through paracrine, autocrine, or intracrine activities either peripherally or in the brain. This has the advantage of limiting the steroid activity to the restricted body regions where the synthesizing cells are located and points to the site-specific feature of neuroactive steroid action.[134-138] It has been suggested that the possible precursors of local neuroactive steroids are DHEA and progesterone.[7] Accordingly, the precise study is necessary about the relationship of dysregulation of neuroactive steroid in oral cavity with the severity and intensity of BMS.

4. Future of BMS research

BMS remains a fascinating, though poorly understood, condition in the field of oral medicine. Several evidence for the neuropathic change as BMS origin is emerging.[3] Recent advances in the etiology, pathophysiology, and management of BMS have lead to an interest in finding diagnostic and prognostic BMS biomarkers.[8-11] The anatomical proximity between saliva and the area of BMS symptoms and the importance of steroid hormones in the pathophysiology of BMS have resulted in the investigation of possible salivary biomarkers.[7]

Research in this area, undertaken according to a variety of approaches, is needed. Thoughtful studies for a clear definition of the associations between BMS and systemic

disorders based on a uniform definition, strict diagnostic criteria, and proper patient selection are also essential. In addition, evidence involving the peripheral and/or central nervous system in BMS should be better documented and confirmed. A caring supportive attitude, a correct patient stratification, and an appropriate multidisciplinary approach will be the gold standards for a rational and beneficial application of current knowledge.

III. MATERIALS AND METHODS

1. Participants

Patients were consecutively recruited among female patients who visited the Department of Oral Medicine, Seoul National University Dental Hospital, with a complaint of burning or painful sensation in the mouth without any visible causative signs. Thirty consecutive female patients with BMS (mean age: 54.5 $\pm$ 6.3 years) were included in this study. All were not smokers. Twenty female volunteers without burning mouth symptoms (mean age: 70.6 $\pm$ 5.7 years) were recruited from social welfare facilities and served as a control group. Exclusion criteria included smoking, oral mucosal pain or diseases, history of treatments for cancer, uncontrolled diabetes, history of taking psychiatric and/or neurologic medications or antihistamines during the past 3 months, and inability to communicate. Clinical evaluation procedures for the control group included oral examination and collection of saliva samples. The research protocol was approved by the Institutional Review Board of the University Hospital (#CRI10032).

2. Clinical evaluation

Clinical evaluation procedures for the patient group included oral examination, interview, panoramic radiography, a questionnaire, a simplified psychological evaluation (symptom checklist-90-revision, SCL-90-R), blood tests, and a measurement of salivary flow rate. The questionnaire was used to evaluate subjective symptoms and included questions about the duration of suffering, area of symptoms, type of discomfort (burning,

aching, stinging, itching, numbness, bad taste, taste alteration, xerostomia, and sore throat), and the effect of oral complaints on daily life (Eff-life). The question - How much do your oral symptoms interfere with your daily life? was asked for the evaluation of Eff-life. The intensities of oral complaints and Eff-life were measured using a visual analog scale (VAS) (0−10 cm, with 10 cm meaning the worst possible).

3. Blood tests

Blood tests were performed to exclude patients with other possible systemic causes of intraoral burning pain or abnormal oral sensation such as anemia, uncontrolled diabetes, and vitamin B_{12} deficiency. The tests included complete blood counts with leukocyte differential counts, erythrocyte sedimentation rate, blood glucose, liver function tests [total protein, albumin, total bilirubin, alkaline phosphatase, aspartate transaminase (AST), alanine transaminase (ALT), and cholesterol], kidney function tests [blood urea nitrogen (BUN) and creatinine], thyroid function tests [triiodothyronine (T3), free thyroxine (T4), and thyroid stimulating hormone (TSH)], calcium, phosphorus, ferritin, vitamin B_{12}, and folate levels.

4. Psychological evaluation

The SCL-90-R was used to evaluate the psychological characteristics of patients. The SCL-90-R is a 90-item self-report measure that has been used to assess psychological symptoms; it comprises nine symptom dimensions, including somatization, obsessive-compulsive, interpersonal sensitivity, anxiety, depression, hostility, phobic anxiety, paranoid ideation, and psychoticism, and three global indices of functioning, including global severity index (GSI), positive symptom distress index (PSDI), and positive symptom total (PST).[139] The GSI is the average score of the 90 items of the questionnaire and is suggested to be the best single indicator of the current level of the disorder. The PST is the number of items scored above zero. The PSDI is the average score of the items scored above zero.

5. Measurement of stimulated and unstimulated whole salivary flow rates

Saliva was collected by a standardized reproducible method. Briefly, samples were collected from the subjects between 09:00 and 11:00 a.m., to minimize diurnal variability. All subjects abstained from eating and drinking for 2 h prior to the measurement of salivary flow rate. Unstimulated whole saliva (UWS) was collected for 10 min by the spitting method. Stimulated whole saliva (SWS) was collected for 5 min by habitual chewing of 1 g of gum base. SWS collected during the first 2 min was discarded. SWS could not be collected from four subjects (two in controls and two in patients) who were unable to chew the gum base because of the loss of posterior teeth. The flow rate of whole saliva was expressed as millilitre per minute. Each saliva sample was collected into a sterile tube and centrifuged at 4000×g for 20 min to remove any cellular debris. The aliquots of the clarified supernatants were stored at -70°C for the experiments.

6. Determination of blood contamination in saliva samples

To determine blood contamination of saliva samples, a salivary blood contamination enzyme immunoassay kit (Salimetrics, State College, PA, USA), which measures the level of transferring in saliva samples, was used. The assay was duplicated, and data were averaged. Subjects having the levels of blood contamination >1.0 ml/dl in SWS were excluded.[140] In the case of UWS, a level of 2.0 ml/dl was used as a guideline because of relatively high levels of blood contamination in UWS.

7. Analysis of salivary cortisol, 17β-estradiol, progesterone, DHEA, and α-amylase

The concentrations of cortisol, 17β-estradiol, progesterone, and DHEA were analyzed from UWS and SWS using enzyme immunoassay kits (Salimetrics). The enzymatic activity of α-amylase from UWS and SWS was determined using a salivary α-amylase assay kit (Salimetrics), which utilizes a chromogenic substrate, 2-chlorop-nitrophenol, linked to maltotriose. All assays were duplicated, and data were averaged. For the duplicated assays of all six items including blood contamination, at least 0.64 ml of saliva sample was needed. In the case of shortage of sample amount owing to a very low salivary flow rate, the

experiments were performed in the order of blood contamination, α-amylase, cortisol, DHEA, progesterone, and 17β-estradiol.

8. Treatment protocol

The flowchart of patient treatment is illustrated in Figure 1. At the first visit, an oral examination, panoramic radiography, and blood test were performed on each patient, and the questionnaire was provided to the patients. At the second appointment, scheduled in the morning, the salivary flow rate was measured, the SCL-90-R was administered, and the questionnaire was checked by the staff to ensure the completion of any omitted sections. Patients were then interviewed by one doctor (HSK) and received an explanation regarding the possible etiology and management strategies for BMS. All patients were provided with the initial treatment with the application of topical lubricant and parafunctional habit control.[141,142] Twenty four patients were re-evaluated using the same questionnaire after 2−4 weeks, and seventeen patients who did not respond to the initial approach were administered clonazepam therapy (0.5 mg clonazepam nightly). If unwanted side effects (in particular, severe drowsiness and/or dizziness) occurred, patients were instructed to take half of a tablet. In this study, three patients experienced unwanted side effects and took half of a tablet. Changes in subjective symptoms were also evaluated after 2−4 weeks using the same questionnaire for the assessment of the efficacy of clonazepam therapy.

9. Statistics

The Kolmogorov−Smirnov normality test was applied to our data. Because the data were not normally distributed, non-parametric tests were used in this study. The Mann−Whitney U-test or Wilcoxon signed rank test was used to determine whether differences existed between or within the groups, respectively. The Spearman correlation analysis was used to determine relationships between the variables.

IV. RESULTS

1. Assessment of BMS questionnaire

To allow for homogeneous grouping of patients with BMS, two non-menopausal patients were excluded from the analyses. Most of the patients (n = 26, 92.9%) complained of oral burning sensations, one patient complained of aching pain, and one patient complained of dysesthesia. The mean duration of oral discomfort was 27.9 months (range 2−180 months). The severity (VAS) of burning pain was 6.86 ± 2.23, and the effect of oral complaints on daily life (Eff-life) was 5.11 ± 2.75. Besides the burning sensation in the oral mucosa, some of the patients reported xerostomia (n = 17, 60.7%) and taste disturbance (n = 18, 64.3%). All the patients complained of a certain discomfort in the tongue area, especially on the tip and lateral borders of the tongue. Some of the patients reported discomfort elsewhere in the oral cavity (lip, n = 6, 21.4%; gingiva／alveolar mucosa, n = 5, 17.9%; palate, n = 5, 17.9%).

Among 28 patients, 22 had history of previous treatments for their burning symptoms. Thirteen patients visited otolaryngology and received various medications including clonazepam, gabapentin, corticosteroids, and gargles. Six visited physicians and received hypnotics or sedatives. Two visited psychiatry and one visited neurology. They also received hypnotics or sedatives. Five visited oriental medicine doctors and received oriental medicines. One visited gynecology and received consultation. Two visited other oral medicine clinics, and one received antifungal medications. One visited oral surgery and received analgesics. Six visited general dental practitioners and received consultation.

2. Comparison between the age-matched patient and control groups

Two patients who were not menopausal and five control subjects showing high blood contamination (≥2.0 mg/dl) in their saliva samples were excluded from analyses, and then, 28 patients (58.8 ± 7.8 years) and fifteen control subjects (68.1 ± 2.3 years) were included the analyses. Among all 43 subjects, the levels of all six analytes including blood

contamination in UWS were significantly correlated with those of SWS ($r_s = 0.467-0.858$). The levels of 17β-estradiol ($r_s = -0.360$, $P < 0.05$), progesterone ($r_s = -0.730$, $P < 0.01$), and DHEA ($r_s = -0.529$, $P < 0.01$) in UWS were significantly correlated with age.

For the age-matched comparison between patient and control groups, 28 patients were divided into two groups according to their ages, the older (≥60 years, mean age: 65.7 ± 4.9 years) and younger (<60 years, mean age: 52.5 ± 2.7 years) groups. Then, fifteen control subjects were compared with the older patient group. The salivary flow rate of UWS was significantly lower in the patient group than in the controls ($P < 0.01$). The patient group also showed a significantly higher levels of cortisol in UWS ($P < 0.05$) and of 17β-estradiol in SWS ($P < 0.05$) (Table 1).

3. Comparison between older and younger patients

The differences in salivary flow rate and levels of salivary steroids and α-amylase between the older and younger patients with BMS were shown in Table 2. Although the older group had low salivary flow rates and high blood contamination levels in UWS, there were no significant differences. The older group showed a significantly lower level of progesterone in UWS ($P < 0.05$). The levels of progesterone ($r_s = -0.581$, $P < 0.01$) and DHEA ($r_s = -0.469$, $P < 0.05$) in UWS were significantly correlated with age.

4. Relationships among the symptoms, the SCL-90-R results, and the levels of salivary analytes of patients

The severity of burning pain or Eff-life showed no significant correlations with the levels of salivary cortisol, 17β-estradiol, progesterone, DHEA, and α-amylase activity. There were no significant correlations between the symptom dimensions and global scales of SCL-90-R and the levels of salivary analytes. The severity of burning pain or Eff-life showed no significant correlations with the results of SCL-90-R (data not shown).

5. Relationships between the levels of salivary analytes and treatment efficacy in the patient group

The severity of initial symptoms and changes in symptoms in the patients following the treatments are shown in Table 3. The severity of burning symptoms decreased significantly following the initial treatment and/or clonazepam therapy. The symptom severity was higher in the patients who did not respond to the initial treatment.

There were no significant correlations (r_s = -0.192-0.307 in burning, r_s = -0.278-0.263 in Eff-life) between the treatment efficacy (decreases of VAS in burning and Eff-life) following the initial treatment and the levels of salivary cortisol, 17β-estradiol, progesterone, DHEA, and α-amylase activity. There were also no significant correlations (r_s = -0.385-0.438 in burning, r_s = -0.331-0.336 in Eff-life) between the treatment efficacy following the clonazepam therapy and the levels of salivary analytes.

V. DISCUSSION

I investigated differences in the levels of salivary cortisol, 17β-estradiol, progesterone, DHEA, and α-amylase activity between patients with BMS and controls and found that the levels of cortisol in UWS and of 17β-estradiol in SWS were significantly higher in patients with BMS. However, the levels of salivary analytes examined could not be predictors of treatment outcome. To the best of our knowledge, this is the first study to consider the level of blood contamination in saliva samples when investigating steroid hormones and α-amylase in patients with BMS. The gingival tissues in the oral cavity usually have certain levels of inflammation, especially in older persons, and therefore, saliva samples usually contain blood contamination. Considering the very big differences in the levels of analytes between blood and saliva, it is very important to exclude saliva samples containing high levels of blood contamination to obtain right answers to the raised hypotheses. In fact, the levels of total protein and pro-inflammatory cytokines in saliva samples are highly correlated with the levels of blood contamination.[125]

Patients with BMS usually report higher levels of psychosocial stress and show

significantly more symptoms of depression and anxiety than controls.[65,143] It is clear that chronic stress and posttraumatic stress disorders, anxiety, and major depression can induce dysfunction of the HPA axis.[7] However, the results of previous studies concerning the level of salivary cortisol in patients with BMS are contradictory. It has been suggested that higher anxiety and salivary cortisol levels are positively associated with the presence of BMS.[10] The results of another study suggested that salivary cortisol levels are not different between patients with BMS and controls, and salivary cortisol levels were found not to be associated with psychosocial stress as measured by the Hospital Anxiety and Depression Scale or the Oral Health Impact Profile quality of life score.[144] These both previous studies did not consider blood contamination of their saliva samples, and the subjects in the latter study were not gender-matched between patients and controls and included male subjects. In fact, after the period of prolonged stress, the factors activating the HPA axis disappeared, but negative feedback suppression of the HPA axis could have persisted and contributed to the reduced cortisol awakening response.[7,145,146] Even though saliva collection was performed between 09:00 and 11:00 a.m. in the present study, the subjects might be at different points in the circadian rhythm of cortisol secretion according to how long after waking up or having sleeping difficulties, which might be related to a large variability of cortisol levels. Despite such complex mechanisms of the HPA axis, our results showed that the level of salivary cortisol is higher in patients with BMS. Further investigations are needed to explain how dysregulation of the HPA axis is involved during the chronic course of BMS pathogenesis.

The possible role of local neuroactive steroids such as DHEA and progesterone in the pathophysiology of BMS has been suggested because of the very restricted location of the burning sensation in the body.[7] The hypothesis was the occurrence of BMS at the time of menopause in women with chronic stress/anxiety disorders was attributed to a fall in gonadal steroids levels concomitantly with a change in adrenal steroid regulation including precursors for neuroactive steroids.[7]

A decreased level of morning salivary DHEA in patients with BMS has been reported.[11] Changes in DHEA in certain diseases or under certain conditions have been explained by its

multifunctional aspects. DHEA could produce neuronal excitability and could increase sensitivity to pain.[147] In contrast, DHEA could exhibit anxiolytic and antidepressive effects.[148,149] The decreased level of salivary DHEA in patients with BMS could thus be accounted for in two ways. The reduction in DHEA could represent an adaptive response to the disorders or could be responsible for aggravation and perpetuation of the symptoms.[11] Contrary to the previous study,[11] the levels of DHEA were higher in our study, although not significantly, in both UWS and SWS in patients with BMS compared with age-matched controls. The increase in salivary DHEA in our study is supported by considering that the main secretagogue for DHEA is ACTH, and DHEA is secreted synchronously with cortisol.[146,150]

Almost all patients with BMS are peri/postmenopausal women. Several lines of evidence have suggested that altered levels of female sex hormones may predispose women to BMS.[3] A higher level of follicular stimulating hormone and a lower level of estradiol have been reported in blood analyses of patients with BMS compared with controls,[8] but the salivary levels of female sex hormones have not been reported in patients with BMS. In this study, the level of 17β-estradiol in SWS was found to be significantly higher in patients with BMS. The levels of 17β-estradiol in UWS and of progesterone in both UWS and SWS were also higher in patients with BMS, although the differences were not significant. Although these results were inconsistent with previous results using blood,[8] possible different adaptive responses of each patient with BMS or possible differences between systemic and local environments could explain our results. In fact, DHEA could be a precursor for sex hormones.[151]

In addition to the long-term dysfunction of the HPA axis and its effects on neural tissues, dysregulation of the sympathetic nervous system can also occur in the presence of chronic stress.[7] For instance, hypocortisolism in patients with stress-related chronic disorders is associated with a hyperactive sympathetic nervous system producing increased catecholamine levels.[152] Salivary α-amylase activity has received increasing attention as a stress marker of the sympathetic nervous system.[24,25] One previous study reported elevated salivary α-amylase levels in patients with oral sensory complains and suggested a

relationship between high levels of α-amylase and increased warm thresholds in patients.[9] However, we did not find any significant differences in the enzymatic activity of α-amylase in both UWS and SWS between patients with BMS and controls.

Most patients with BMS suffer from chronic pain conditions and experience diminished quality of life. Many patients spend lots of time visiting clinicians in diverse specialties. Clinicians usually attempt various treatments including medications and behavioral therapies, but there is no single treatment modality or medication that is effective for most patients with BMS. As a result, clinicians are usually faced with difficulties when selecting treatment options and predicting treatment efficacy. It is therefore critical to identify an effective mode of treatment and to find factors for prognosis prediction.[20] We have reported clinical outcome predictors in previous studies.[20,142] The psychological status and initial symptom severity were outcome predictors for the initial treatment and/or clonazepam therapy in patients with BMS. However, in this study, we were unable to identify salivary markers related to treatment outcome in patients with BMS. There were no significant correlations among the symptom severity, the symptom dimensions and global scales of SCL-90-R, and the levels of salivary analytes in patients with BMS. However, our results showed that the levels of all salivary analytes were increased, although not always significantly, in patients with BMS, indicating that dysregulations of the HPA axis and gonadal steroids are involved in the pathogenesis of BMS.

VI. CONCLUSIONS

BMS is characterized by a painful burning sensation or other dysesthesias of the oral mucosa, with no visible mucosal abnormalities upon clinical examination, so that seriously exacerbates quality of life. The anatomical proximity between saliva and the area of BMS symptoms and the importance of steroid hormones in the pathophysiology of BMS have resulted in the investigation of possible salivary biomarkers. The aim of this study was to investigate salivary cortisol, 17β-estradiol, progesterone, DHEA and α-amylase levels in

patients with BMS compared with controls and to investigate whether these levels could be predictors for treatment outcome in patients with BMS. Thirty female patients with BMS and twenty female control subjects were included. UWS and SWS samples were collected, and their flow rates were determined. Salivary levels of cortisol, 17β-estradiol, progesterone and DHEA were analyzed using enzyme immunoassay kits. The enzymatic activity of α-amylase was determined using maltotriose as a substrate. Salivary transferrin level was measured to determine the level of blood contamination in saliva samples. SCL-90-R was used for psychological characteristics of patients with BMS. Treatment protocols of patients with BMS included control of parafunctional habits, use of artificial saliva, and clonazepam medication.

The obtained results were as follows:

1. The patient group showed significantly higher levels of cortisol in UWS ($P < 0.05$) and of 17β-estradiol in SWS ($P < 0.05$).

2. When the patients were divided into older (≥ 60 years) and younger (<60 years) groups, the older group showed a significantly lower level of progesterone in UWS ($P < 0.05$).

3. There was no significant correlation between all scales of SCL-90-R and the levels of salivary analytes.

4. There was no significant correlation between the treatment efficacy and the levels of salivary analytes.

In conclusion, patients with BMS had significantly higher levels of cortisol in UWS and

of 17β-estradiol in SWS. These indicate that dysregulations of the HPA axis and gonadal steroids are involved in the pathogenesis of BMS.

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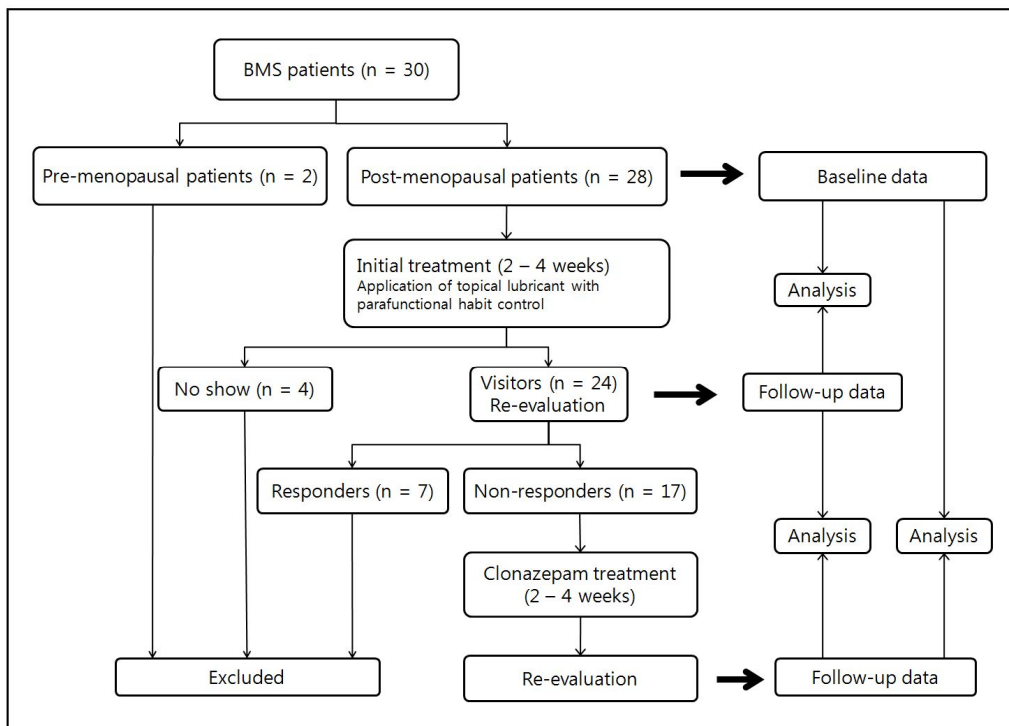
Figure Legend

Fig. 1. Patient flow diagram

Fig. 1

Table 1. Age, salivary flow rate, salivary levels of blood contamination, cortisol, 17β-estradiol, progesterone, DHEA, and salivary α-amylase activity in age-matched BMS patients and controls.

		Controls (n = 15)	Patients (n = 14)	P-value[a]
		Mean ± SD	Mean ± SD	
Age (years)		68.1 ± 2.3	65.7 ± 4.9	0.102
Flow rate (ml/min)	UWS	0.15 ± 0.10 (n = 15)	0.08 ± 0.10 (n = 14)	0.008
	SWS	0.63 ± 0.49 (n = 15)	0.46 ± 0.25 (n = 12)	0.648
Blood contamination[b] (mg/dl)	UWS	1.06 ± 0.57 (n = 15)	1.18 ± 0.46 (n = 14)	0.533
	SWS	0.66 ± 0.42 (n = 15)	0.61 ± 0.25 (n = 12)	0.943
Cortisol (μg/dl)	UWS	0.237 ± 0.319 (n = 15)	0.263 ± 0.102 (n = 13)	0.029
	SWS	0.169 ± 0.098 (n = 15)	0.232 ± 0.094 (n = 12)	0.114
17β-Estradiol (pg/ml)	UWS	2.009 ± 1.037 (n = 13)	2.289 ± 0.424 (n = 8)	0.064
	SWS	2.447 ± 0.621 (n = 15)	3.217 ± 0.942 (n = 12)	0.014
Progesterone (pg/ml)	UWS	38.3 ± 13.4 (n = 14)	55.4 ± 29.9 (n = 10)	0.154
	SWS	37.9 ± 9.9 (n = 15)	39.2 ± 11.6 (n = 12)	0.943
DHEA (pg/ml)	UWS	70.1 ± 39.8 (n = 15)	102.6 ± 43.8 (n = 11)	0.077
	SWS	65.8 ± 37.2 (n = 15)	75.2 ± 52.8 (n = 12)	0.981
α-Amylase activity (U/ml)	UWS	158.7 ± 86.1 (n = 15)	184.3 ± 225.7 (n = 14)	0.451
	SWS	139.8 ± 73.6 (n = 15)	154.1 ± 83.1 (n = 12)	0.581

BMS, burning mouth syndrome; UWS, unstimulated whole saliva; SWS, stimulated whole saliva; DHEA, dehydroepiandrosterone

[a]The Mann-Whitney U-test statistics for comparison

[b]The level of blood contamination was determined by measuring the transferrin level in saliva samples.

Table 2. Age, salivary flow rate, salivary levels of blood contamination, cortisol, 17β-estradiol, progesterone, DHEA, and salivary α-amylase activity in BMS patients according to age.

		< 60 years (n = 14)	≥ 60 years (n = 14)	P-value[a]
		Mean ± SD	Mean ± SD	
Age (years)		52.5 ± 2.7	65.7 ± 4.9	0.000
Flow rate (ml/min)	UWS	0.15 ± 0.13 (n = 14)	0.08 ± 0.10 (n = 14)	0.062
	SWS	0.51 ± 0.31 (n = 14)	0.46 ± 0.25 (n = 12)	0.742
Blood contamination[b] (mg/dl)	UWS	0.87 ± 0.38 (n = 13)	1.18 ± 0.46 (n = 14)	0.054
	SWS	0.64 ± 0.31 (n = 14)	0.61 ± 0.25 (n = 12)	0.820
Cortisol (μg/dl)	UWS	0.313 ± 0.220 (n = 12)	0.263 ± 0.102 (n = 13)	0.979
	SWS	0.289 ± 0.222 (n = 14)	0.232 ± 0.094 (n = 12)	0.940
17β-Estradiol (pg/ml)	UWS	3.210 ± 2.835 (n = 11)	2.289 ± 0.424 (n = 8)	0.904
	SWS	3.031 ± 1.651 (n = 14)	3.217 ± 0.942 (n = 12)	0.193
Progesterone (pg/ml)	UWS	91.9 ± 39.9 (n = 11)	55.4 ± 29.9 (n = 10)	0.036
	SWS	44.0 ± 25.2 (n = 14)	39.2 ± 11.6 (n = 12)	0.899
DHEA (pg/ml)	UWS	162.1 ± 83.7 (n = 12)	102.6 ± 43.8 (n = 11)	0.190
	SWS	105.1 ± 69.1 (n = 14)	75.2 ± 52.8 (n = 12)	0.252
α-Amylase activity (U/ml)	UWS	164.3 ± 112.4 (n = 13)	184.3 ± 225.7 (n = 14)	0.685
	SWS	153.4 ± 71.1 (n = 14)	154.1 ± 83.1 (n = 12)	0.860

BMS, burning mouth syndrome; UWS, unstimulated whole saliva; SWS, stimulated whole saliva; DHEA, dehydroepiandrosterone

[a]The Mann-Whitney U-test statistics for comparison

[b]The level of blood contamination was determined by measuring the transferrin level in saliva samples.

Table 3. The severity of baseline symptoms and change in symptoms following treatments in patients with BMS.

	Baseline (Group I)	After initial treatment (Group II)	After clonazepam treatment (Group III)	Significance[a]
Burning (VAS)	6.90 ± 2.16 (n = 30)[b]			
	6.86 ± 2.23 (n = 28)[c]			
	6.85 ± 2.24 (n = 24)[d]	4.69 ± 2.72 (n = 24)[d]		***(I,II)
	7.38 ± 1.58 (n = 17)[e]	5.68 ± 2.27 (n = 17)[e]	4.91 ± 2.27 (n = 17)[e]	***(I,III)**(I,II)*(II,III)
Eff-life (VAS)	5.05 ± 2.80 (n = 30)[b]			
	5.11 ± 2.75 (n = 28)[c]			
	4.90 ± 2.73 (n = 24)[d]	3.92 ± 2.90 (n = 24)[d]		N.S.
	5.15 ± 2.31 (n = 17)[e]	4.71 ± 2.75 (n = 17)[e]	3.97 ± 3.21 (n = 17)[e]	N.S.

BMS, burning mouth syndrome; VAS, visual analog scale; Eff-life, the effect of oral complaints on daily life

[a]The Wilcoxon-signed ranked test statistics for comparison

[b]The total patients included

[c]Post-menopausal patients

[d]Patients re-evaluated following the initial treatment

[e]Patients re-evaluated following both the initial treatment and clonazepam therapy

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

국문초록

구강작열감증후군 환자의 타액 Cortisol, 17β-Estradiol, Progesterone, Dehydroepiandrosterone, α-Amylase 연구

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　　구강작열감증후군은 구강점막의 뚜렷한 병변 없이 혀를 포함한 구개면, 입술점막 등 구강점막 전체 또는 일부의 작열감 및 감각이상을 초래하여 삶의 질을 심각하게 저하시키는 질환이다. 구강작열감증후군 증상은 타액이 존재하는 구강에 한정되어 발생하고 병태생리학적으로 스테로이드 호르몬이 중요하게 고려되고 있으므로 타액 표지자 연구가 주목을 받고 있다. 본 연구의 목적은 구강작열감증후군 환자의 타액 cortisol, 17β-estradiol, progesterone, dehydroepiandrosterone (DHEA), α-amylase 농도를 대조군과 비교하고, 이러한 물질이 치료 결과를 예측할 수 있는 표지자로 사용될 수 있는지를 조사하는 것이다. 구강작열감증후군 환자군 30명, 정상 대조군 20명을 연구대상으로 하여, 비자극시 전타액과 자극시 전타액을 채취하였고 타액 분비율을 측정하였다. 타액 cortisol, 17β-estradiol, progesterone 및 DHEA 농도는 효소면역법을 활용하여 측정하였고, α-amylase 효소 활성은 maltotriose를 기질로 사용하여 분석하였으며, 타액의 혈액오염도 분석을 위해 타액 transferrin 농도를 측정하였다. 또한 구강작열감증후군 환자의 정신 · 심리학적 특성과 타액 분석물과의 연관성을 관찰하기 위해 간이정신진단검사 (Symptom Checklist-90-Revision, SCL-90-R) 를 시행하였다. 이와 더불어 구강작열감증후군 환자의 치료 효과와 타액

분석물과의 상관관계를 알아보기 위해 이상기능습관 조절, 인공타액 처방 및 clonazepam 투여 후 그 변화를 관찰하였으며 다음과 같은 결과를 얻었다.

1. 환자군에서 비자극시 전타액의 cortisol 농도 ($P < 0.05$) 와 자극시 전타액의 17β-estradiol 농도 ($P < 0.05$) 가 유의하게 높았다.

2. 환자군을 고연령층 (60세 이상) 과 저연령층 (60세 미만) 으로 나누었을 때, 고연령층에서 비자극시 전타액의 progesterone 농도가 유의하게 낮았다 ($P < 0.05$).

3. 타액 분석물 농도와 SCL-90-R의 모든 항목간에는 유의한 상관관계가 나타나지 않았다.

4. 타액 분석물 농도와 구강작열감증후군 치료 효과 사이에는 유의한 상관관계가 나타나지 않았다.

이상의 결과는 구강작열감증후군 환자에서 비자극시 전타액의 cortisol 농도와 자극시 전타액의 17β-estradiol 농도가 유의하게 높게 나타났다. 이는 구강작열감증후군의 병태생리학적 기전에 hypothalamic-pituitary-adrenal axis와 여성 호르몬의 조절 장애가 관련되어 있음을 제시해 준다.