치의과학 박사학위논문

Relationships between Oral MUC1 Expression and Salivary Hormones in Burning Mouth Syndrome

구강작열감증후군 환자의 구강점막 상피세포 MUC1의 발현과 타액 스트레스 호르몬 및 여성 호르몬 농도의 상관관계

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2017년 6월

위 원 장 _____(인)

부 위 원 장 _____(인)

위 원 _____(인)

위 원 _____(인)

Abstract

Relationships between Oral MUC1 Expression and Salivary Hormones in Burning Mouth Syndrome

Kang, Jeong-Hyun, D.D.S., M.S.D.
Program in Oral Medicine and Oral Diagnosis
Department of Dental Science
Graduate School, Seoul National University
(Directed by Professor Hong-Seop Kho, D.D.S., M.S.D., Ph.D.)

Objectives: Burning mouth syndrome (BMS) is a complex disease whose etiopathophysiology is still vague, but dysregulated psychoendocrinological mechanisms and altered oral mucosal integrity have been regarded as significant factors. The aim of this study was to investigate the relationships among oral mucosal epithelial MUC1 expression, salivary female gonadal hormones, stress markers, and oral symptoms in BMS patients.

Materials and Methods: Thirty post-menopausal female patients with BMS (60.0 ± 5.0 years) were included. Clinical and psychological evaluations were performed and the expression level of oral mucosal epithelial MUC1 was analyzed. The levels of cortisol, dehydroepiandrosterone (DHEA), 17β-estradiol, progesterone, chromogranin A, and blood contamination were determined from unstimulated whole saliva (UWS) and stimulated whole saliva (SWS) samples.

Results: Salivary progesterone level had significant positive correlations with oral mucosal epithelial MUC1 expression level and with salivary cortisol and DHEA levels. The salivary level of 17β-estradiol showed significant positive correlations with period of symptom duration, severity of effects of oral complaints on daily life, and results from psychological evaluations. Cortisol level in UWS and cortisol/DHEA ratio in UWS and SWS had negative correlations with severity of

oral burning sensation significantly. The severity of taste disturbance had positive correlations with results from psychometry significantly.

Conclusions: Dysregulated psychoendocrinological interactions might affect oral mucosal MUC1 expression and severity of oral burning sensation in post-menopausal BMS patients.

Keywords: MUC1; Stress; Gonadal hormone; Saliva; Burning mouth syndrome

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Jeong-Hyun Kang, D.D.S., M.S.D.

Program in Oral Medicine and Oral Diagnosis

Department of Dental Science, Graduate School, Seoul National University

(Directed by Professor **Hong-Seop Kho,** D.D.S., M.S.D., Ph.D.)

강 정 현

서울대학교 대학원 치의과학과 구강내과·진단학 전공

(지도교수 고 홍 섭)

-CONTENTS-

Korean abstract

I. Introduction

Burning mouth syndrome (BMS), a chronic oral mucosal pain disorder, is characterized by discomforts in the form of burning and/or stinging sensations that affect the oral mucosa without objective signs to justify the symptoms (Lopez-Jornet et al., 2010; Scala et al., 2003). BMS mainly occurs in peri- or post-menopaual women, and its etiology is multifactorial. Many physical and psychological conditions such as parafunctional habits, local nerve trauma, salivary gland dysfunctions, hormonal changes after menopause, depression, and anxiety have been reported as potential predisposing factors (Bergdahl & Bergdahl, 1999; Patton et al., 2007; Scala et al., 2003).

Recent evidence has indicated central and/or peripheral neuropathic changes as the most significant etiopathogenic mechanisms in BMS development (Mendak-Ziółko et al., 2012; Puhakka et al., 2016; Yilmaz et al., 2007). Repetitive local irritations to the oral mucosa, oral parafunctional habits, reduction of salivary output, and changes in salivary composition can lead to peripheral neuropathic changes in oral mucosal tissues (Gorsky et al., 1991; Grushka & Sessle, 1991; Ko et al., 2011; Paterson et al., 1995). Decreased oral mucosal integrity resulting from repetitive local stimuli and decreased lubrication capacity in the oral cavity might underly the occurrence of oral burning pain.

The epithelia of the oral mucosa is continuously exposed to local injuries. Salivary glycoproteins such as mucins are crucial molecules for protecting the oral mucosa from these irritations by forming a protective cover on the oral mucosal surfaces (Slomiany et al., 1996). Secretory salivary mucins such as MUC5B and

MUC7 protect the oral mucosa indirectly by interacting with oral microorganisms and concentrating antimicrobials in mucin network close to the oral mucosal surfaces (Wickström et al., 2000). Oral epithelial surface-bound mucin molecules, MUC1, protect the oral mucosa more directly (Gendler, 2001). MUC1, ubiquitously present in the glandular and luminal lining surfaces (Gendler, 2001; Hattrup & Gendler, 2008), possesses extended negatively-charged carbohydrate chains that protect the oral mucosa by forming network structures with secretory mucins and other proteins (Gibbins et al., 2015; Kullaa et al., 2014; Pramanik et al., 2010).

Decreased expression of oral mucosal epithelial MUC1 in older adults compared to young ones have been reported (Chang et al., 2011). Interestingly, however, oral mucosal epithelial MUC1 expression was higher in post-menopausal BMS patients than age- and sex-matched controls (Kho et al., 2013). The adaptation of oral mucosal tissues to repetitive irritation has been suggested as a possible mechanism of the increase in oral MUC1 expression, but it remains to be answered whether psychoendocrinological dysregulations commonly found in BMS patients could affect oral MUC1 expression. Female gonadal hormones and stress-related biomarkers are known to be dysregulated in BMS patients (Kim et al., 2012; Woda et al., 2009). Although the results have not been consistent, altered levels of female gonadal hormones such as 17ß-estradiol, progesterone, and follicle-stimulating hormone (FSH) in serum and saliva have been found in patients with BMS (Dias Fernandes et al., 2009; Gao et al., 2009; Kim et al., 2012). Altered levels of cortisol and dehydroepiandrosterone (DHEA), which reflect stressful and/or adverse life events, have also been reported (Amenabar et al., 2008; Dias

Fernandes et al., 2009; Kim et al., 2012).

The relationships between oral MUC1 expression and salivary gonadal and stress hormones in healthy young female adults throughout the menstrual cycle were investigated (Lee et al., 2015) and significant negative correlation between oral mucosal epithelial MUC1 expression and cortisol/DHEA ratio were found. A significant negative correlation was also found between oral MUC1 expression and progesterone, especially during the mid-luteal phase, demonstrating that changes in gonadal and stress hormones affect the oral mucosal defense. The present study was to extend previous findings about psychoendocrinological influences on oral mucosal defense to patients with BMS. The hypothesis was that dysregulated gonadal and stress hormones related to the development of BMS affect the increased oral mucosal epithelial MUC1 expression previously reported in patients with BMS.

II. Literature review

1. Burning mouth syndrome (BMS)

BMS is a condition featured by subjective feeling described by the patient as pain and/or discomforts such as burning and stinging which occurred in the oral cavity, without clinical or laboratory evidences to justify these symptoms (Lopez-Jornet et al., 2010; Scala et al., 2003). Classification of BMS are based on the clinical findings that 'primary BMS', in which the causes could not be clarified, and 'secondary BMS', attributed from local or systemic factors (Scala et al., 2003).

Various factors could be involved in etiopathogenesis of burning sensations in the oral cavity, and these factors have been divided into local, systemic, and psychological categories. Repetitive local irritations to the oral mucosa could cause peripheral neuropathic changes in oral mucosal tissues (Gorsky et al., 1991; Grushka & Sessle, 1991; Ko et al., 2011; Paterson et al., 1995).

The relationships between BMS and systemic factors such as nutritional deficiencies and hormonal changes have been referred in previous reports (Femiano et al., 2008; Hugoson & Thorstensson, 1991; Lamey & Lamb, 1988; Moore et al., 2007). Decreased level of vitamin B_{12}, ferritin, and folate had been found in BMS patients, and after prescribing supplement of vitamin B and ferrous sulphate, the symptoms alleviated (Hugoson & Thorstensson, 1991; Lamey & Lamb, 1988). Hormonal changes may have roles in occurrence of burning pain in the oral cavity. One study revealed that hypothyroidism could be suspicious cause of oral burning and dysguesia (Feminio et al., 2008). Another study focused on the role of diabetes mellitus in peripheral neuropathy in patients with type 1 diabetes and burning sensation and the results suggested that a neuropathic process may be an underlying source of BMS (Moore et al., 2007).

It is well established that BMS patients showed higher levels of somatization, anxiety, or depression compared to controls (de Souza et al., 2011; Eli et al., 1994; Schiavone et al., 2012). However, the relationships between the extents of pain suffering and the results from psychometry have not shown consistency (Schiavone et al., 2012). One previous study mentioned that the psychometry data correlated with only the degree of affective pain, not that of

sensory pain in BMS patients (Carlson et al., 2000). These studies imply that psychopathologic factors may have an important role in BMS and support the multifactorial etiology, by interacting with physical changes.

The primary location of the symptom is the tongue, and pain from BMS is characterized by continuous and spontaneous intense burning sensation in the oral cavity (Lopez-Jornet et al., 2010; Patton et al., 2007). Two specific clinical features such as dysguesia and xerostomia, usually occurs accordance with burning symptoms and these three symptoms are described as “symptomatic triad” (Scala et al., 2003).

Many investigations have focused on the alteration in taste perception which might be related with burning pain. About 70% BMS patients were suffered from persistent taste disturbance (Femiano et al., 2008; Imura et al., 2016), and the altered taste was usually bitter and/or metallic (Ship et al., 1995). Both disturbance in the taste perception and burning pain might be a result of dysregulated sensory modalities at the level of small-diameter afferent fibers (Ship et al., 1995).

Because no valid and standardized criteria used to diagnose BMS has been established yet, the true prevalence is uncertain. From National Health Interview survey in 1989, prevalence of burning sensation in the oral cavity in the US civilians age 18 and over was identified as 0.7% of all adults (Lipton et al., 1993). The condition predominantly affects women, with a relation of approximately 3:1 compared to men. BMS is observed principally in middle-aged patients, particularly in postmenopausal women (Bergdahl & Bergdahl, 1999; Lamey & Lamb, 1988). Occurrence under 30 years is rare and never having been found in children or adolescent (van der Waal, 1990). These differences seem

mainly due to the biological, psychological, and sociocultural differences, but the pathophysiology of BMS is still controversial.

Because the pathophysiology of burning symptom is controversial, multidisciplinary approach is recommended for searching for management methods. If the burning pain is attributed from certain causes, such as Candidal infection, vitamin deficiency, anemia and so on, the first approach should try to alleviate these suspicious factors. However these kinds of treatments are usually not satisfactory. Then other medications such as anti-depressant, topical capsaicin, alpha-lipoic acid and so on could be considered. The most-used medication for treatment of BMS is clonazepam. The efficacy of topical (Gremeau-Richard et al., 2004; Woda et al., 1998) and systemic clonazepam (Grushka et al., 1998) has been mentioned previously. Gabapentin, another anti-psychotic drug and alpha lipoic acid, a powerful neuroprotector seemed to have little effects in some patients with BMS but not in others (López-D'alessandro & Escovich, 2011). The tricyclc antidepressants such as nortriptyline and amitriptyline, serotonin inhibitors, and sertraline also have reported that these medication have effects in alleviating symptoms in the BMS patients (Lopez-Jornet et al., 2010).

The etiology of BMS is multifactorial and has not been fully understood yet. To approach the development of standardized diagnostic protocol and strategies for management, interdisciplinary research should be required.

2. Menopause and gonadal hormonal changes

2.1. Menopause

As life expectancy increase, a number of women who experienced menopause have been increased. The World Health Organization has defined the menopause as the complete cessation of menstruation attributed to loss of ovarian follicular activity (World Health Organization, 1996). Other terms, such as perimenopause, which refers to time starting a few years prior and sustained after the occurrence of menopause, and climacteric, that means time after the termination of reproductive function are used.

The median age of beginning menopause has been mentioned as 45.7 years (McKinlay et al., 1992) in women in United States, and as 48 years in Korean women (Hong et al., 2006). The primary determinant factor of age of menopause is genetics, but it is thought that general health status also could affect the timing of menopause. Smoking seemed to be related with onset of menopause 1 to 2 years earlier (McKinlay et al., 1992). Greater body mass has been thought to be related with delayed menopause (McKinlay et al., 1992), and malnourishment and vegetarianism have been thought to be associated with earlier onset of menopause (Biela, 2002). Furthermore women with shorter menstrual cycle showed earlier menopause, and multigravida women or those who used oral contraceptives showed later menopause (Harlow & Signorello, 2000).

The menopausal transition is accompanied with many biological, physical and neuroendocrinological changes. Many symptoms and signs are related to female gonadal hormonal deficiency, and merge with issues of natural aging. For example, the risk of cardiovascular diseases during postmenopausal period increases and the rate of bone loss is accelerated (Chahal & Drake, 2007; Riggs &

Melton, 1986). Moreover hot flush and cognitive disturbance could occur (Hammar et al., 1998; Shaywitz et al., 1999). Hormone replacement therapies were developed to reduce these symptoms, and some of them were effective (American College of Physicians, 1992).

As the aging population and life expectancy increases, medical care targeted to postmenopausal women becomes an important issues of future medicine and dentistry. Further research and clinical consideration about this would be needed for researchers and clinicians.

2.2. Female gonadal hormonal changes

2.2.1. Estradiol

The Stages of Reproductive Aging Workshop demonstrated simplified reproductive aging stages in 2012 (The Stages of Reproductive Aging Workshop +10, STRAW + 10) (Harlow et al., 2012). According to this system, during early (stage -2) and late (stage -1) menopausal transition stages, shortened menstrual cycle length and elevated concentrations of follicular stimulating hormone (FSH) and low Anti-Mullerian hormone (AMH) are detected, and functional capacity of the ovary is also decreased (Harlow et al., 2012). The elevation in FSH concentrations could be detected several years before menopause and continues increasing 2 years after menopause (stage +1). On the other hand, estradiol levels decrease during stage -2 to stage +1 and are stabilized about 2 years after menopause because functional capacity of the ovary is decreased as aging process of reproductive system of women proceeds (Randolf et al., 2011). Elevated

gonadotropin levels are sustained due to reduced secretion of estratiol (Randolf et al., 2011).

The most remarked hormonal changes during perimenopause are significant reduction in estradiol (Yen, 1977). The estrone, a conjugate form of estrogen which acts as circulating reservoir of estradiol is less reduced than estradiol because estrone is produced mainly by peripheral aromatization from androgen, not only by ovary (Yen, 1977).

Abundant human research has suggested that ovarian failure induce the menopause. Eventually follicular activity ceases, estrogen concentration fall and other gonadotropins rise accordance with decreased estrogen levels. Thus the changes of levels of estradiol would have key role to understand hormonal changes during menopause.

2.2.2. Progesterone

In perimenopausal women, the ovary shows reduced number of follicles, and the hormonal changes of older women are related with the depletion of ovarian follicles. In the early perimenopausal stage, the concentration of estradiol was reduced, but normal luteal phase concentration of progesterone was observed (Sherman et al., 1976). In later stages, diminished secretions of both estradiol and progesterone were detected (Sherman et al., 1976).

Anovulation increases as women progress through the transition to menopause. Several studies focused on the relationships between longer

anovulation, declining luteal secretion, and decreased levels of pregnanolone, the inactive metabolite of progesterone (O'Connor et al., 2009; te Velde & Pearson, 2002). Thus gamatogenic ovarian failure may lead to decreased secretion of progesterone and decreased levels of progesterone or its metabolite may have a role in increased anovulation and declining luteal secretion.

3. Stress markers

3.1. Cortisol

Cortisol is one of the main stress hormones which mediate stress and physiological body responses. Cortisol is synthesized by cholesterol in the zona fasciculata of human adrenal gland cortex. The secretion and biological response of cortisol is regulated by hypothalamic-pituitary-adrenal (HPA) axis. Under stress, the medial parvocellar region in the hypothalamus release corticotropin-releasing hormone (CRH), and CRH binds to receptors in the anterior pituitary to promote the production of adrenocorticotropic hormone (ACTH) (Herman & Cullinan, 1997). Circulating ACTH is main regulator of glucocorticoid, mainly cortisol in the adrenal gland cortex. Secretory cortisol finally maintains whole body homeostasis and the organisms' response to stress. The negative feedback of HPA axis on the ACTH secretory response confines the duration of the tissue exposure to cortisol, and minimize the catabolic, anti-reproductive, and immunosuppressive effects of cortisol (Tsigos & Chrousos, 2002).

Under acute stress, an increased level of cortisol induces catabolic processes to provide more energy to the tissue, and increased cortisol levels return

to the basal levels by negative feedback of HPA axis. On the contrary, under chronic stress, results are heterogeneous with studies, reporting blunted as well as heightened cortisol levels or no differences in the HPA axis response (Kudielka et al., 2009; Lightman. 2008). It is generally accepted that exposure to stress can cause heightened cortisol levels. If higher cortisol level is prolonged for a while, elevated levels of cortisol could damage on hippocampal and cortical neurons and finally dysregulate the HPA axis mechanisms (Sapolsky et al., 1986). Thus when researching the mechanisms of chronic pain, dysregulated HPA axis should be considered seriously.

3.2. Dehydroepiandrosterone (DHEA)

DHEA and its sulphate form (DHEA-S) are sex steroid precursors, and secreted by the zona fasciculate layer of adrenal cortex. Though concentration of DHEA-S are much higher than concentration of DHEA due to increased half-life and decreased clearance rate of DHEA-S than that of DHEA, only desulphated form of DHEA is biologically active and DHEA-S is regarded as reservior of DHEA.

The secretion rates of DHEA and DHEA-S are controlled by CRH and ACTH. The changes of levels of DHEA under acute or chronic stress have been reported several times in previous studies. The elevated concentration of DHEA under acute physiologic and psychosocial stress has been reported (Lennartsson et al., 2012; Oberbeck et al., 1998). On the contrary, chronic stress seems to be associated with unchanged or decreased DHEA concentrations (Oberbeck et al.,

1998). Interestingly, dissociation of adrenal DHEA and cortisol release during chronic stress is observed (Parker et al., 1985) and this may imply that DHEA secretion is controlled independently from cortisol.

Previous studies demonstrated that DHEA levels declined about 30-50% in age 50s compared to those of in 30s. This might be due to the decreased mass of the zona reticularis (Kushnir et al., 2010; Parker et al., 1985). The hypothesis that the stress-induced DHEA increase is also decreased at older age could be deduced, and one study showed that administration of ACTH or CRH lead to elevate cortisol levels in same extents in both old and young subjects but the increase in DHEA and DHEA-S was much less in old subjects (Vermeulen et al., 1982).

3.3. Cortisol/DHEA ratio

Despite the same site of origin and being induced by a similar set of hormones, cortisol and DHEA mediate opposing biological and neurologic functions. Because DHEA has anabolic and anti-glucocorticoid effects and shows adverse effects of cortisol, the ratio of cortisol and DHEA means the equilibrium between catabolic and anabolic activities, and has been regarded as indicator of "functional cortisol effect".

Under acute or chronic stress, cortisol/DHEA ratio shows different patterns. Under acute stress, DHEA elevation was paralleled by increased cortisol levels, but the peak of DHEA preceded that of cortisol (Izawa et al., 2008; Oberbeck et al., 1998). On the contrary, if stress sustained for a while, cortisol level was decreased, DHEA level showed blunted response, and cortisol/DHEA, an indicator of the

'functional cortisol effect', was decreased (Gallagher et al., 2016; Mouthaan et al., 2014). Thus cortisol/DHEA could be a better marker of severity of symptoms than cortisol or DHEA level alone in patients under chronic stress.

3.4. Chromogranin A (CgA)

CgA is an acidic glycoprotein which is secreted accordance with catecholamine from the adrenal medulla and the sympathetic nerve endings (Takiyyuddin et al., 1994). Numerous pairs of amino acids consist of CgA structures. After cleavage by the prohormone convertases of the neurosecretory granules, the proteolytic fragments of CgA such as catestatin, vasostatins, pancreastatin, and parastatin finally have broad range of regulatory activities on the cardiovascular, endocrine, and immune systems (Helle et al., 2007).

CgA has been regarded as valid indicator of sympathoadrenal activity. Moreover concentration of CgA in saliva was elevated under conditions of stress (Nakane et al., 2002). Salivary CgA has not been regarded as originating from blood but rather is released from the submandibular gland duct into saliva by autonomic stimulation (Saruta et al., 2005). The durability and small time lag regarded as advantages of salivary CgA as a biomarker of mental stress (Gallina et al., 2011), but the levels of CgA could be affected by symptoms of oral dryness or reduced salivary flow rate and showed circadian variations as those of cortisol (Den et al., 2007). Thus when using salivary CgA as a stress marker, the conditions in oral cavities of the subjects and sampling time should be considered.

4. Oral mucosal defense and MUC1

Mucins are defined as glycoproteins containing 50-90% of their molecular sizes as oligosaccharides (Gendler, 2001). There are two types of mucins. Firstly, secreted mucins are the front line of protection for epithelial surface, acting as physical covers between extracellular environment and mucosal surface by gel-forming. Secretory salivary mucins such as MUC5B and MUC7 protect the oral mucosa indirectly by interacting with oral microorganisms and concentrating antimicrobials in mucin network close to the oral mucosal surfaces (Wickström et al., 2000). Another type of mucins are tethered transmembrane mucins which are regarded as next line of defense. This type of mucins act like sensor of any disturbance to the cell surfaces and signals this information to the inside of the cell (Gendler, 2001). MUC1 is a second type of mucin which is a single pass transmembrane protein with a heavily glycosylated extracellular domain that extends up to 200-500 μm from the cell surface (Gendler, 2001; Hattrup & Gendler, 2008). MUC 1 is routinely expressed in the glandular and luminal epithelial lining cells of the mammary gland, esophagus, stomach, duodenum, pancreas, uterus, prostate, lung, and oral cavity. (Gendler, 2001; Hattrup & Gendler, 2008). The extended negatively charged sugar chains of MUC1 make physical covers and provide an anti-adhesive property to MUC1 so confining accessibility from pathogen and preventing pathogenic colonization, resulting in lubricating and protecting the underlying epithelia from both physical and chemical irritations (Kufe, 2009; Schroten et al., 1992; Yolken et al., 1992). MUC1 in the oral cavity protect the oral mucosa more directly by forming network structures with secretory mucins and other proteins (Gibbins et al., 2015; Kullaa et al., 2014; Pramanik et al.,

2010).

Several factors have been suggested as having a role in controlling the amount of epithelial MUC1 expression in the oral cavity. Higher amount of oral MUC1 expression was detected in adolescent with higher frequency of dental caries (Gabryel-Porowska et al., 2014). Moreover BMS patients showed higher oral mucosal epithelial MUC1 expression than age- and sex-matched controls (Kho et al., 2013), and healthy young women showed different patterns of oral MUC1 expressions throughout their menstrual cycles and stages (Lee et al., 2015). Thus it seems that the adaptation mechanisms of oral mucosal tissues from local irritation and endocrinological regulations have roles in controlling the amount of oral MUC1 expressions, but still the specific mechanisms remain controversial.

5. Saliva as a diagnostic fluid

Saliva is a complex mixture composed of secretions from major and minor salivary glands and several other constituents, such as gingival crevicular fluid, microorganisms, desquamated epithelial cells, and food debris. Saliva has been considered as ‘a mirror of body’ because variety of analytes from systemic sources could reach to the oral cavity through various pathways. Even though blood and urine have been used as the most widely used laboratory setting diagnostic fluid, whole saliva has been considered as an alternative of blood and urine because of its non-invasive and relatively simple collecting procedures (Malamud, 1993). Saliva contains various biomarkers which make it possible for bioassays developed as point-of-care device such as detection of human

immunodeficiency virus, human papillomavirus, hepatitis C virus antibodies, and RNA from Zika virus (Corstjens et al., 2016; Priye et al., 2017). Furthermore many commercial kits for monitoring hormones and lipid soluble drugs are also available and have been applied in everyday clinics (Chiappin et al., 2007; Saxena and Kumar, 2015; Wood, 2009). The rapid detections of other life-threatening diseases such as cancers, kidney diseases, Alzheimer's disease, Parkinson's diseases, and oral cancer by saliva have been also applied (Master et al., 2015; Peng et al., 2013; Shi et al., 2011; Xiao et al., 2015).

Saliva has several advantages and disadvantages compared to urine and blood such as easy and non-invasive collecting procedures. However saliva has disadvantages such as wide normal range of analytes, low analytes concentration and lack of standardized procedures. Levels of analytes in plasma such as steroid hormones usually show up to 10 folds higher concentration than those in saliva (Chiappin et al., 2007; Nune et al., 2015). Because of higher levels of analytes in serum compared to those in saliva, if blood leakage reaches a certain threshold, the salivary levels of analytes should be artificially elevated (Kivlighan et al., 2004; Schwartz and Granger, 2004).

Early studies of salivary diagnosis focused the biomarkers of specific disease in saliva without understating of origin of these biomarkers. However nowadays advances in oral fluid biomarkers have been extremely developed by molecular biological knowledge and advanced experimental technologies. The future research about this field would try to improve diagnostic efficacy of saliva and widen the spectrum of diagnosis to other systemic diseases in a point of care.

III. Materials and methods

1. Participants

Thirty-seven post-menopausal women were recruited from patients who attended the Department of Oral Medicine (Seoul National University Dental Hospital, Seoul, South Korea) with complaints of a burning sensation and dysesthesia in the oral cavity without objective clinical abnormality between 1 Nov., 2014 and 30 Sep., 2015. Exclusion criteria for the participants were; smoker, having uncontrolled diabetes, having a history of malignant cancer, having a history of beam radiation and/or radioisotope treatment in the head and neck region, a medication history of immunosuppressants and/or cytotoxic drugs, and being incapable of communication.

Of thirty-seven participants, two patients were excluded due to high levels of blood contamination in their saliva samples. Because hormone levels in the blood are much higher than those in the saliva, the high amount of blood in saliva samples lead to incorrect results. The cut-off values were 2.0 mg/dL of transferrin in the case of unstimulated whole saliva (UWS) and 1.0 mg/dL of transferrin in stimulated whole saliva (SWS) (Schwartz & Granger, 2004). One patient was excluded because she became menopause one year before participation. It has been reported that gonadal hormonal levels reach stabilization about at least two years after menopause (Harlow et al., 2012). One patient was excluded because of low serum vitamin B_{12} level and high serum fasting glucose level, and another due to

decreased serum ferritin and hemoglobin levels. One patient withdrew without specific reason, leaving a final total of thirty patients (mean age, 60.0 ± 5.0 years) in the study. The research protocol was reviewed in compliance with the Helsinki Declaration and approved by the Institutional Review Board of the Seoul National University Dental Hospital (#CRI14036) in 10 Oct., 2014 and informed consents were obtained from all participants.

2. Clinical evaluation

Clinical evaluation procedures for the participants included oral examination, panoramic radiography, psychological evaluation [Symptom Checklist-90-Revised (SCL-90-R)] (Derogatis & Cleary, 1977), and salivary flow rate measurement. A questionnaire was used to evaluate subjective discomforts, and consisted of questions about duration and areas of symptoms, characteristics of discomfort (burning, aching, stinging, itching, numbness, taste disturbance, dry mouth, and sore throat), and the effect of oral complaints on daily life (Eff-life). The severities of oral discomforts and Eff-life were scored by a visual-analog-scale (VAS) (0-10 cm, with 10 cm indicating the worst imaginable discomfort). Oral parafunctional habits, including pressing the tongue against the teeth, tongue or cheek biting, diurnal tooth clenching, and nocturnal clenching or bruxing were also recorded.

3. Psychological evaluation

The SCL-90-R was used to examine the psychological status of the participants. The SCL-90-R is a tool for evaluating psychological symptoms by analyzing the answers to 90 questions, and provides results of the nine symptom dimensions, such as somatization (SOM), obsessive-compulsive (O-C), interpersonal sensitivity (I-S), depression (DEP), anxiety (ANX), hostility (HOS), phobic anxiety (PHOB), paranoid ideation (PAR), and psychoticism (PSY). Three global functioning indices, such as global severity index (GSI), positive symptom distress index (PSDI), and positive symptom total (PST) were also provided.

4. Blood tests

Blood tests were performed to rule out other possible systemic factors that may cause oral burning pain and/or abnormal oral sensations. The tests included complete blood counts with leukocyte differential and hematinic-related components such as iron, ferritin, vitamin B_{12}, and folate. Blood glucose, liver function tests (total protein, albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and cholesterol), kidney function tests (blood urea nitrogen and creatinine), and thyroid function tests [T3 (triiodothyronine), free T4 (thyroxine), and TSH (thyroid-stimulating hormone)] were also performed. In addition, calcium, phosphorus, magnesium, and zinc levels were analyzed.

5. Collection of saliva samples

Saliva was collected between 9:00 a.m. and 11:00 a.m. to minimize variability due to circadian rhythm. The participants reported wake-up times from 5:30 a.m. to 7:30 a.m., and the mean time interval between waking-up and saliva collection was 3.3 ± 1.1 hours. Eating and drinking were prohibited for 2 hours prior to the saliva sampling. UWS was collected for 15 min by drooling the saliva into a tube. Mechanical stimulation, chewing of 1 g of gum base, was used to stimulate salivary flow. The saliva collected for the first 2 min was discarded, and then SWS was collected for the next 10 min with the mechanical chewing stimulation. Salivary flow rate was expressed as mL/min.

Two milliliters of SWS from each participant were added to RNA stabilizing solution (Oragene RNA RE-100, DNA Genotek Inc., Ottawa, ON, Canada) and used for RNA extraction. The UWS and remnant SWS samples were centrifuged at 10,000 xg for 20 min at 4°C to remove any cellular debris. The clarified supernatants were aliquoted and frozen at -70°C for analytic experiments.

6. Determination of oral mucosal epithelial MUC1 expression level

Total RNA was extracted from the mixture of Oragene RNA RE-100 (DNA Genotek Inc.) and SWS sample using a RNeasy Mini kit (Qiagen, Hilden, Germany). The concentration of RNA in each sample was determined at 260 nm using a spectrophotometer (Nanodrop 1000, Thermo Fisher Scientific, Wilmington, DE, USA). The mean amount of total RNA was 24.2 ± 14.6 μg. The absorbance

ratio at A260/280 of each sample was 1.8 - 2.0, and the extracted RNA samples were stored at -70°C.

The real-time PCR experiments were performed to quantify MUC1 mRNA levels relative to ß-actin and GAPDH mRNA levels, as described in previous reports (Kho et al., 2013; Lee et al., 2015). Briefly, quantitative PCR reactions were performed with an ABI 7500 (Applied Biosystems, Foster City, CA, USA) using TaqMan probes (Applied Biosystems) and cDNA synthesized using 1 μg of total RNA. The crossing points of MUC1 with both ß-actin and GAPDH were applied to the formula, $2^{-(\text{MUC1-ß-actin(or GAPDH)})}$, to calculate the relative level of MUC1 to both housekeeping genes. Finally, the averaged fold ratios from both housekeeping genes were used as the relative MUC1 mRNA level. Each experiment was performed in duplicate. Two reactions, one without template and one without reverse transcriptase, were also performed as negative controls.

7. Determination of cortisol, DHEA, 17ß-estradiol, progesterone, chromogranin A, and blood contamination in saliva samples

The concentrations of cortisol, DHEA, 17ß-estradiol, and progesterone were analyzed from UWS and SWS by enzyme immunoassays (Salimetrics, State College, PA, USA). Cortisol/DHEA ratio was obtained by dividing the concentraion of cortisol by that of DHEA in the same unit. Chromogranin A (CgA) level was also determined using an immunoassay (Yanaihara institute Inc.,

Shizuoka, Japan). To determine the level of blood contamination in saliva samples, an immunoassay measuring the concentration of transferrin in saliva was used (Salimetrics, State College, PA, USA). All assays were duplicated and data were averaged. In the UWS of one participant, analyses of 2 items, DHEA and CgA, could not be performed due to a shortage of sample.

Intra-assay and inter-assay coefficients of variation for the cortisol assay were 3.0% and 3.0%, respectively, and its sensitivity was 0.007 μg/dL. Those for DHEA were 5.6% and 8.2%, and its sensitivity was 5.0 pg/mL, those for 17ß-estradiol were 8.1% and 8.9%, and its sensitivity was 0.1 pg/mL, those for progesterone were 6.2% and 7.6%, and its sensitivity was 5.0 pg/mL, those for CgA were 10.5% and 13.3%, and its sensitivity was 0.14 pmol/mL, those for blood contamination were 4.9% and 7.1%, and its sensitivity was 0.08 mg/dL. The cross-reactivity of the cortisol assay with cortisone was 0.13%.

8. Statistics

Based on the Kolmogorov-Smirnov normality test, data in this study were normally distributed; therefore, parametric tests were applied. The paired T-test and Pearson's correlation analysis were used. All tests were two-sided and *P*-values less than 0.05 were considered statistically significant.

IV. Results

1. BMS questionnaires

Most patients (n = 28, 93.3%) complained of oral burning symptoms. Two patients who did not complain of burning pain reported an itching sensation and an aching type of pain, respectively. Aching sensation was the second most common type of pain and was reported by 18 patients (60.0%). Twenty patients (66.7%) complained of oral dryness and 18 (60.0%) complained of taste disturbance. The mean duration of discomforts was 28.1 ± 39.3 months. The severity (VAS score) of burning sensation was 6.1 ± 2.9 and that of Eff-life was 4.4 ± 3.0. All patients reported the tongue as the main symptom area, especially the tongue tip (n = 23, 76.7%). Other than the tongue, pain was reported in the lip by nine patients, the palate by seven patients, and the gingiva by five patients. Pressing the tongue against the teeth was the most common habit reported by the patients (n = 8, 26.7%) (Table 1).

2. Salivary flow rate and SCL-90-R

The mean flow rate of whole saliva in the unstimulated state was 0.27 ± 0.17 mL/min and that in the stimulated condition by gum chewing was 1.11 ± 0.48 mL/min (Table 1). According to the SCL-90-R results, the T-scores of all nine symptom dimensions were in the range of 30 - 60 in all patients except two. In one patient, the T-score was higher than 60 in SOM and in another patient, T-score were higher than 60 in SOM, O-C, DEP, and ANX (data not shown).

3. Salivary hormone and blood contamination levels

The mean concentrations of almost every salivary analyte in UWS tended to be higher than those in SWS, but only mean levels of progesterone, CgA, and blood contamination were significantly different between UWS and SWS (Table 2).

4. Correlations among levels of salivary analytes and severities of oral symptoms

The level of 17β-estradiol in UWS had a significant positive correlation with period of symptom duration (r = 0.626, $P < 0.001$) and that in SWS showed a significant positive correlation with VAS score of Eff-life (r = 0.397, $P = 0.030$). The cortisol level in UWS had a significant negative correlation with severity of burning sensation (r = -0.391, $P = 0.032$), but the DHEA levels in both UWS and SWS did not show any statistical significances with severity of burning sensation. Cortisol/DHEA in both UWS (r = -0.517, $P = 0.004$) and SWS (r = -0.401, $P = 0.028$) had also significant negative correlations with severity of burning pain (Table 3).

5. Correlations among levels of salivary analytes and oral mucosal MUC1 expression

Correlations between all variables were analyzed in both UWS and SWS. The most prominent finding was that the levels of progesterone in UWS (r = 0.550,

$P = 0.002$) and SWS (r = 0.414, $P = 0.023$) showed significant positive correlations with the level of oral mucosal MUC1 expression. Progesterone levels also had positive correlations with cortisol and DHEA levels significantly, with higher level of significance between progesterone and DHEA than between progesterone and cortisol. Cortisol/DHEA showed significant negative correlations with DHEA levels in both UWS (r = -0.633, $P < 0.001$) and SWS (r = -0.626, $P < 0.001$), but not significant positive correlations with cortisol levels. In addition, the levels of 17β-estradiol showed significant positive correlations with the level of progesterone in UWS (r = 0.418, $P = 0.022$) and with the level of DHEA in SWS (r = 0.363, $P = 0.049$) (Tables 4 and 5).

Concentrations of all analytes except CgA were not affected by the blood contamination levels in either UWS or SWS. The levels of CgA had significant positive correlations with the levels of blood contamination both in UWS (r = 0.571, $P = 0.001$) and SWS (r = 0.440, $P = 0.015$). Salivary flow rates did not affect the levels of salivary analytes, except for the level of CgA in SWS (r = -0.439, $P = 0.015$) (Tables 4 and 5).

6. Correlations among symptoms, levels of salivary analytes, and SCL-90-R results

The severity of burning pain did not correlate with the results of the psychometry, but the VAS scores of aching sensation (r = 0.380, $P = 0.038$) and Eff-life (r = 0.377, $P = 0.040$) had positive correlations with the score of HOS

significantly. The duration of symptoms also had a positive correlation with the score of O-C (r = 0.363, P = 0.049). Interestingly, there were significant positive relationships between the severity of taste disturbance and the results of psychometry, for DEP (r = 0.492, P = 0.006), ANX (r = 0.418, P = 0.022), PHOB (r = 0.407, P = 0.026), PSY (r = 0.383, P = 0.037), and GSI (r = 0.412, P = 0.024). The severity of numbness followed, with significant positive correlations with the scores of PHOB (r = 0.751, P < 0.001), GSI (r = 0.361, P = 0.049), and PST (r = 0.443, P = 0.014) (Supplementary Table S1).

17β-Estradiol was the only salivary gonadal hormone that had significant correlations with the results of the psychometry. The levels of 17β-estradiol in both UWS (r = 0.412, P = 0.024) and SWS (r = 0.503, P = 0.005) showed positive correlations with the score of O-C significantly. The level of 17β-estradiol in SWS also had positive correlations with the scores of DEP (r = 0.420, P = 0.021), PSY (r = 0.461, P = 0.010), GSI (r = 0.397, P = 0.030), and PST (r = 0.399, P = 0.029). The level of CgA in SWS showed negative correlations with the scores of I-S (r = -0.386, P = 0.035), HOS (r = -0.475, P = 0.008), and PAR (r = -0.367, P = 0.046) (Supplementary Table S2).

V. Discussion

Several studies have tried to explain the etiopathogenic mechanisms of BMS based on altered levels of gonadal and stress hormones and oral MUC1 expression pattern. It has been reported that BMS patients had elevated levels of

FSH and cortisol and decreased level of estradiol in their blood samples (Amenabar et al., 2008; Gao et al., 2009). On the other hand, BMS patients had increased 17β-estradiol and cortisol levels and decreased DHEA level in their saliva samples compared to age- and sex-matched controls (Dias Fernandes et al., 2009; Kim et al., 2012). Given that patients with BMS with abnormal gonadal and stress hormone levels also had higher level of oral mucosal MUC1 expression compared to elderly subjects without oral burning pain (Chang et al., 2011; Kho et al., 2013), the focus of this study was the influences of dysregulated gonadal and stress hormones on oral mucosal MUC1 in patients with BMS.

The effects of gonadal hormones such as progesterone on uterine MUC1 expression related with embryo implantation have been reported (Dharmaraj et al., 2010; Meseguer et al., 2001). However, there has been only one previous report on the influence of gonadal and stress hormones on oral mucosal MUC1, featuring young healthy females throughout their menstruation cycles (Lee et al., 2015). That study found that the expression level of oral mucosal epithelial MUC1 was negatively correlated with salivary cortisol/DHEA throughout the whole menstrual cycle and was also negatively correlated with salivary cortisol, cortisol/DHEA, and progesterone, especially during the mid-luteal phase (Lee et al., 2015). These findings imply that stress-related endocrinological interactions decrease oral mucosal MUC1 expression in healthy young female adults.

The novel findings of this study were that the level of salivary progesterone showed significant positive correlations with the levels of oral mucosal MUC1 expression and salivary cortisol in BMS patients, in contrast to

results from previous studies. A relationship between progesterone and cortisol has been previously reported (Liening et al., 2010; Reinfenstein, 1946; Wirth et al., 2007). The concept that stress and increased activity of the hypothalamic-pituitary-adrenal (HPA) axis have the potential to inhibit the hypothalamic-pituitary-gonadal (HPG) axis has been widely accepted (Reifenstein, 1946). In menopausal BMS patients with sustained oral pain under ceased reproductive function, the effects of stress on the adrenal glands would be much greater than those on the gonadal glands. The adrenal glands could secrete progesterone as an important intermediary precursor in synthesis of the adrenal corticoids (bartosuk et al., 1957), at a rate controlled by adrenocorticotropic hormone (ACTH) (Holzbauer & Newport, 1969). Thus, under conditions of stress, ACTH might up-regulate the secretion of progesterone as well as cortisol in the adrenal gland. In the same manner, a positive correlation between salivary levels of progesterone and cortisol has also been observed in men and women taking hormonal oral contraceptives (Liening et al., 2010; Wirth et al., 2007). The mechanisms underlying the positive correlation between progesterone and oral mucosal MUC1 could not be fully explained. Increased oral mucosal MUC1 expression could be the result of adaptation in the oral mucosal tissues to repetitive oral parafunction and chronic local irritation (Kho et al., 2013), which might be related to inability to cope with increased psychological stress. However, hormonal changes related to psychological stress might affect the level of oral mucosal MUC1 expression. Further research about psychoendocrinological influences on oral mucosal tissues at the molecular level is needed.

The involvement of 17β-estradiol in the subjective pain perception

process could be inferred from its positive correlations with period of symptom duration, severity of Eff-life, and results from the psychological evaluation in the present study. Though the exact role of estrogen in pain cognition is inconclusive, the results of many studies have indicated that female gonadal hormones are involved in pain modulation. The effects of endogenous hormones have been investigated by studies on gender differences in pain perception or menstrual-related changes of pain (Ge et al., 2006; Mathew et al., 2013). The effects of exogenous hormones have been investigated by studies on changes of pain perception in post-menopausal women by hormone replacement therapy (Fillingim & Edwards, 2001). Both kinds of studies showed that increase or fluctuation of estrogen level is related to decrease of pain thresholds and increase of pain perception. Estrogen is also known to affect emotion. Estrogen replacement therapy has been suggested to improve psychological function and general well-being in postmenopausal women (Ditkoff et al., 1991). However, a significant increase in negative mood and anxiety has been reported in postmenopausal women following estrogen administration (Newhouse et al., 2008), suggesting that the impact of estrogen on psychological status is not straightforward and may vary depending on the situation. Therefore, it could be suggested that the altered level of 17β-estradiol in patients with BMS might be associated with the subjective awareness of oral discomforts and altered psychological status.

The negative correlations between cortisol or cortisol/DHEA and severity of burning sensation imply an effect of stress on the subjective awareness of discomforts in patients with BMS. In the acute stress phase, cortisol and DHEA levels are elevated, with the peak of DHEA preceding that of cortisol (Izawa et al.,

2008; Vermes & Beishuizen, 2001). However, in subjects under sustained stress, cortisol level was decreased, DHEA level showed blunted response, and cortisol/DHEA, an indicator of the 'functional cortisol effect', was decreased (Gallagher et al., 2016; Mouthaan et al., 2014). The results of this study indicate that psychoendocrinological interactions affect the severity of symptoms in patients with BMS, and that cortisol/DHEA could be a better marker of severity of symptoms than cortisol or DHEA level alone in patients with BMS.

Neurological interactions between pain and taste perceptions are well known (Bartoshuk et al., 2005), and taste disturbance is a common symptom in patients with BMS (Femiano et al., 2008; Imura et al., 2016). The association of taste disturbance with psychological conditions, including stress, depression, and especially anxiety has been reported (Bergdahl & Bergdahl, 2002; Davies et al., 2016), which supports the positive correlations between taste disturbance and psychometry in the present study. Taste thresholds and perceptions have been reported to be affected by the level of cortisol changed by circadian rhythms or acute stress in healthy subjects (Al'Absi et al., 2012; Fujimura et al., 1990). However, a relationship between salivary cortisol level and severity of taste disturbance was not found in the present study. Occurrence and severity of taste disturbance are thought to be affected by complex psychoneuroendocrinological interactions, not just cortisol level in post-menopausal BMS patients with a dysregulated HPA axis.

It is well known that psychologic indices such as somatization, anxiety, or depression tend to be higher in BMS patients (Eli et al., 1994; de Souza et al., 2011;

Schiavone et al., 2012). However, the relationships between the degree of pain suffering and the results of psychometry are not always consistent, which may depend on the type of psychometry tools (Schiavone et al., 2012). It has also been reported that the psychometry data correlated with only the affective pain rating, not the sensory pain rating in BMS patients (Carlson et al., 2000). Above results showed no significant correlations between the severity of burning pain and the results of the psychometry. These results could be affected by the facts that the T-scores of psychometry in the BMS patients included in this study were not very high and we evaluated the sensory pain rating.

There are limitations in the present study. First, because of lack of a control group, the results could not show the degree of abnormalities in stress and gonadal homonal levels and patterns of MUC1 expression in patients with BMS. However, based on the findings from previous reports, it could be hypothesized the existence of relationships between altered gonadal and/or stress hormonal levels and oral mucosal MUC1 expression patterns in patients with BMS. Thus, it is thought that the lack of a control group does not critically lessen the value of this study. Second, as relatively small numbers of subjects were included and the levels of correlation coefficients were relatively weak and moderate, the influences of confounding factors should be considered when interpreting the results.

In conclusion, the etiology of BMS is multifactorial and its pathophysiology involves complex psychoneuroendocrinological parameters. The results of the present study showed that altered stress-related endocrinological interactions in post-menopausal BMS patients might affect the expression of oral

MUC1 and occurrence of oral burning sensations.

References

Al'Absi, M., Nakajima, M., Hooker, S., Wittmers, L., Cragin, T. (2012). Exposure to acute stress is associated with attenuated sweet taste. *Psychophysiology,* 49, 96-103.

Amenabar, J.M., Pawlowski, J., Hilgert, J.B., Hugo, F.N., Bandeira, D., Lhuller, F., Lopes de Souza, M.A. (2008). Anxiety and salivary cortisol levels in patients with burning mouth syndrome: case-control study. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics,* 105, 460-465.

American College of Physicians. (1992). Guidelines for counseling postmenopausal women about preventive hormone therapy. American College of Physicians. *Annals of Internal Mededicine,* 15, 1038-1041.

Balfour, W.E., Comline, R.S., Short, R.V. (1957). Secretion of progesterone by the adrenal gland. *Nature*, 180, 1480-1481.

Bartoshuk, L.M., Snyder, D.J., Grushka, M., Berger, A.M., Duffy, V.B., Kveton, J.F. (2005). Taste damage: previously unsuspected consequences. *Chemical Senses,* 30, i218-i219.

Biela, U. Determinants of the age at natural menopause. (2002). *Przeglad Lekarski,* 59, 165-169.

Bergdahl, M., Bergdahl, J. (1999). Burning mouth syndrome: prevalence and associated factors. *Journal of Oral Pathology and Medicine,* 28, 350-354.

Bergdahl, M., Bergdahl, J. (2002). Perceived taste disturbance in adults: prevalence and association with oral and psychological factors and medication. *Clinical Oral Investigations,* 6, 145-149.

Carlson, C.R., Miller, C.S., Reid, K.I. (2000). Psychosocial profiles of patinets with burning mouth syndrome. *Journal of Orofacial Pain*, 14, 59-64.

Chahal, H.S., Drake, W.M. (2007). The endocrine system and ageing. *The Journal of Pathololgy,* 211, 173-180.

Chang, W.I., Chang, J.Y., Kim, Y.Y., Lee, G., Kho, H.S. (2011). MUC1 expression in the oral mucosal epithelial cells of the elderly. *Archives of Oral Biology,* 56, 885-890.

Chiappin, S., Antonelli, G., De Palo, E.F. (2007). Saliva specimen: A new laboratory tool for diagnostic and basic investigation. *Clinical Chimica Acta,* 383, 30-40.

Corstjens, P.L., Abrams, W.R., Malamud, D. (2016). Saliva and viral infections. *Periodontololgy 2000,* 70, 93-110.

Davies, S.J., Underhill, H.C., Abdel-Karim, A., Christmas, D.M., Bolea-Alamanac, B.M., Potokar, J., Herrod, J., Prime, S.S. (2016). Individual oral symptoms in burning mouth syndrome may be associated differentially with depression and anxiety. *Acta Odontologica Scandinavia,* 74, 155-160.

de Souza, F.T., Teixeira, A.L., Amaral, T.M., dos Santos, T.P., Abreu, M.H., Silva, T.A., Kummer, A. (2012). Psychiatric disorders in burning mouth syndrome. *Journal of Psychosomatic Research,* 72, 142-146.

Den, R., Toda, M., Nagasawa, S., Kitamura, K., Morimoto, K. (2007). Circadian rhythm of human salivary chromogranin A. *Biomedical Research,* 28, 57-60.

Derogatis, L.R., Cleary, P.A. (1977). Factorial invariance across gender for the primary symptom dimensions of the SCL-90. *British Journal of Social Clinical*

Psychology, 16, 347-356.

Dharmaraj, N., Wang, P., Carson, D.D. (2010). Cytokine and progesterone receptor interplay in ther regulation of MUC1 gene expression. *Molecular Endocrinology,* 24, 2253-2266.

Dias Fernandes, C.S., Salum, F.G., Bandeira, D., Pawlowski, J., Luz, C., Cherubini, K. (2009). Salivary dehydroepiandrosterone (DHEA) levels in patients with the complaint of burning mouth: a case-control study. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics,* 108, 537-543.

Ditkoff, E.C., Crary, W.G., Cristo, M., Lobo, R.A. (1991). Estrogen improves psychological function in asymptomatic postmenopausal women. *Obstetrics & Gynecology,* 78, 991-995.

Eli, I., Kleinhauz, M., Baht, R., Littner, M. (1994). Antecedents of burning mouth syndrome (glossodynia) - recent life events vs. psychopathologic aspects. *Journal of Dental Research,* 73, 567-572.

Fillingim, R.B., Edwards, R.R. (2001). The association of hormone replacement therapy with experimental pain responses in postmenopausal women. *Pain,* 92,

229-234.

Femiano, F., Lanza, A., Buonaiuto, C., Gombos, F., Nunziata, M., Cuccurullo, L., Cirillo, N. (2008). Burning mouth syndrome and burning mouth in hypothyroidism: proposal for a diagnostic and therapeutic protocol. *Oral Surgery Oral Medicine Oral Pathololog*y *Oral Radiology and Endodontology,* 105, e22-e27.

Fujimura, A., Kajiyama, H., Tateishi, T., Ebihara, A. (1990). Circadian rhythm in recognition threshold of salt taste in healthy subjects. *American Journal of Physiology,* 259, R931-R935.

Gabryel-Porowska, H., Gornowicz, A., Bielawska, A., Wójcicka, A., Maciorkowska, E., Grabowska, S.Z., Bielawski, K. (2014). Mucin levels in saliva of adolescents with dental caries. *Mededical Science Monitor,* 18, 72-77.

Gallagher, S., Sumner, R.C., Muldoon, O.T., Creaven, A.M., Hannigan, A. (2016). Unemployment is associated with lower cortisol awakening and blunted dehydroepiandrosterone responses. *Psychoneuroendocrinology*, 17, 41-49.

Gallina, S., Di Mauro, M., D'Amico, M.A., D'Angelo, E., Sablone, A., Di Fonso, A., Bascelli, A., Izzicupo, P., Di Baldassarre, A. (2011). Salivary chromogranin A, but

not α-amylase, correlates with cardiovascular parameters during high-intensity exercise. *Clinical Endocrinolology (Oxford)* 75, 747-752.

Gao, J., Chen, L., Zhou, J., Peng, J. (2009). A case-control study on etiological factors involved in patients with burning mouth syndrome. *Journal of Oral Pathology and Medicine,* 38, 24-28.

Ge, H.Y., Madeleine, P., Cairns, B.E., Arendt-Nielsen, L. (2006). Hypoalgesia in the referred pain areas after bilateral injections of hypertonic saline into the trapezius muscles of men and women: a potential experimental model of gender-specific differences. *The Clinical Journal of Pain*, 22, 37-44.

Gendler, S.J. (2001). MUC1, the renaissance molecule. *Journal of Mammary Gland Biology and Neoplasia*, 6, 339-353.

Gibbins, H.L., Proctor, G.B., Yakubov, G.E., Wilson, S., Carpenter, G.H. (2015). sIgA biding to mucosal surfaces in mediated by mucin-mucin interactions. *PLoS One*, 10, e0119677.

Gorsky, M., Silverman, S., Jr., Chinn, H. (1991). Clinical characteristics and management outcome in the burning mouth syndrome. An open study of 130

patients. *Oral Surgery, Oral Medicine, Oral Pathology,* 72, 192-195.

Gremeau-Richard, C., Woda, A., Navez, M.L., Attal, N., Bouhassira, D., Gagnieu, M.C., Laluque, J.F., Picard, P., Pionchon, P., Tubert, S. (2004). Topical clonazepam in stomatodynia: a randomised placebo-controlled study. *Pain,* 108, 51-57.

Grushka, M., Sessle, B.J. (1991). Burning mouth syndrome. *Dental Clinics of North America,* 35, 171-184.

Grushka, M., Epstein, J., Mott, A. (1998). An open-label, dose escalation pilot study of the effect of clonazepam in burning mouth syndrome. *Oral Surgery Oral Medicine Oral Patholology Oral Radiology and Endodontology,* 86, 557-561.

Harlow, S.D., Gass, M., Hall, J.E., Lobo, R., Maki, P., Rebar, R.W., Sherman, S., Sluss, P.M., de Villiers, T.J., Group, S.C. (2012). Executive summary of the stages of reproductive aging workshop + 10: addressing the unfinished agenda of staging reproductive aging. *The Journal of Clinical Endocrinology & Metabolism,* 97, 1159-1168.

Hammar, M., Christau, S., Nathorst-Böös, J., Rud, T., Garre, K. (1998). A double-blind, randomised trial comparing the effects of tibolone and continuous combined

hormone replacement therapy in postmenopausal women with menopausal symptoms. *An International Journal of Obstetrics & Gynecology,* 105, 904-911.

Hattrup, C.L., Gendler, S.J. (2008). Structure and function of the cell surface (tethered) mucins. *Annual Review of Physiology,* 70, 431-457.

Harlow, B.L., Signorello, L.B. (2000) Factors associated with early menopause. *Maturitas,* 28, 3-9.

Helle, K.B., Corti, A., Metz-Boutigue, M.H., Tota, B. (2007). The endocrine role for chromogranin A: a prohormone for peptides with regulatory properties. *Cellular and Mollecular Life Science,* 64, 2863-2886.

Herman, J.P., Cullinan, W.E. (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends in Neurosciences,* 20, 78-84.

Holzbauer, M., Newport, H.M. (1969). Adrenal secretion rates and adrenal tissue concentrations of pregnenolone, progesterone, 11 beta OH-androstenedione and some other steroids in young pigs and dogs. *The Journal of Physiology,* 200, 821-848.

Hong, J.S., Yi, S.W., Kang, H.C., Jee, S.H., Kang, H.G., Bayasgalan, G., Ohrr, H. (2007). Age at menopause and cause-specific mortality in South Korean women: Kangwha Cohort Study. *Maturitas,* 20, 411-419.

Hugoson, A., Thorstensson, B. (1991). Vitamin B status and response to replacement therapy in patients with burning mouth syndrome. *Acta Odontologica Scandinavia* 49, 367-375.

Imura, H., Shimada, M., Yamazaki, Y., Sugimoto, K. (2016). Characteristic changes of saliva and taste in burning mouth syndrome patients. *Journal of Oral Pathology and Medicine,* 45, 231-236.

Izawa, S., Sugaya, N., Shirotsuki, K., Yamada, K.C., Ogawa, N., Ouchi, Y., Nagano, Y., Suzuki, K., Nomura, S. (2008). Salivary dehydroepiandrosterone secretion in response to acute psychosocial stress and its correlation with biological and psychologic changes. *Biological Psychology,* 79, 294-298.

Kho, H.S., Chang, J.Y., Kim, Y.Y., Kim, Y. (2013). MUC1 and Toll-like receptor-2 expression in burning mouth syndrome and oral lichen planus. *Archives of Oral Biology,* 58, 837-842.

Kim, H.I., Kim, Y.Y., Chang, J.Y., Ko, J.Y., Kho, H.S. (2012). Salivary cortisol, 17β-estradiol, progesterone, dehydroepiandrosterone, and alpha-amylase in patients with burning mouth syndrome. *Oral Diseases,* 18, 613-620.

Kivlighan, K.T., Granger, D.A., Schwartz, E.B., Nelson, V., Curran, M., Shirtcliff, E.A. (2004). Quantifying blood leakage into the oral mucosa and its effects on the measurement of cortisol, dehydroepiandrosterone, and testosterone in saliva. *Hormones and Behavior,* 46, 39-46.

Ko, J.Y., Park, I.H., Park, H.K., Kho, H.S. (2011). Outcome predictors of initial treatment with topical lubricant and parafunctional habit control in burning mouth syndrome (BMS). *Archives of Gerontology and Geriatrics,* 53, 263-269.

Kudielka, B.M., Hellhammer, D.H., Wüst S. (2009). Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. *Psychoneuroendocrinology* 34, 2-18.

Kufe, D.W. (2009). Mucins in cancer: function, prognosis and therapy. *Nature Review Cancer,* 9, 874-885.

Kullaa, A.M., Asikainen, P., Herrala, M., Ukkonen, H., Mikkonen, J.J. (2014). Microstructure of oral epithelial cells as an underlying basis for salivary mucosal

pellicle. *Ultrastructural Pathology,* 38, 382-386.

Kushnir, M.M., Blamires, T., Rockwood, A.L., Roberts, W.L., Yue, B., Erdogan, E., Bunker, A.M., Meikle, A.W. (2010). Liquid chromatography-tandem mass spectrometry assay for androstenedione, dehydroepiandrosterone, and testosterone with pediatric and adult reference intervals. *Clinical Chemistry,* 56,1138-1147.

Lamey, P.J., Lamb, A.B. (1988) Prospective study of aetiological factors in burning mouth syndrome. *British Medical Journal (Clinical Research Ed),* 30, 1243-1246.

Lee, Y.H., Kim, Y.Y., Chang, J.Y., Kho, H.S. (2015). Changes in oral mucosal MUC1 expression and salivary hormones throughout the menstrual cycle. *Oral Diseases,* 21, 962-968.

Lennartsson, A.K., Kushnir, M.M., Bergquist, J., Jonsdottir, I.H. (2012). DHEA and DHEA-S response to acute psychosocial stress in healthy men and women. *Biological Psychology,* 90,143-149.

Liening, S.H., Stanton, S.J., Saini, E.K., Schultheiss, O.C. (2010). Salivary testosterone, cortisol, and progesterone: two-week stability, interhormone correlations, and effects of time of day, menstrual cycle, and oral contraceptive use

on steroid hormone levels. *Physiology & Behavior,* 99, 8-16.

Lightman, S.L. (2008). The neuroendocrinology of stress: a never ending story. *Journal of Neuroendocrinology,* 20, 880-884.

Lipton, J.A., Ship, J.A., Larach-Robinson, D. (1993). Estimated prevalence and distribution of reported orofacial pain in the United States. *The Journal of American Dental Association* 124, 115-121.

Lopez-Jornet, P., Camacho-Alonso, F., Andujar-Mateos, P., Sanchez-Siles, M., Gomez-Garcia, F. (2010). Burning mouth syndrome: an update. *Medicina Oral, Patologia Oral y Cirugia Bucal*, 15, e562-e568.

López-D'alessandro, E., Escovich, L. (2011), Combination of alpha lipoic acid and gabapentin, its efficacy in the treatment of Burning Mouth Syndrome: a randomized, double-blind, placebo controlled trial. *Medicina Oral, Patologia Oral y Cirugia Bucal*, 16, e635-e-640.

Malamud, D. (2011). Saliva as a diagnostic fluid. *Medical Clinics of North America,* 55, 159-178.

Masters, J.M., Noyce, A.J., Warner, T.T., Giovannoni G., Proctor G.B. (2015). Elevated salivary protein in Parkinson's disease and salivary DJ-1 as a potential marker of disease severity. *Parkinsonism & Related Disorders* 21, 1251-1255.

Mathew, P.G., Dun, E.C., Luo, J.J. (2013). A cyclic pain: the pathophysiology and treatment of menstrual migraine. *Obstetrical & Gynecological Survey,* 68, 130-140.

McKinlay, S.M., Brambilla, D.J., Posner, J.G. (1992). The normal menopause transition. *Maturitas,* 14, 103-15.

Mendak-Ziółko, M., Konopka, T., Bogucki, Z.A. (2012). Evaluation of select neurophysiological, clinical and psychological tests for burning mouth syndrome. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology,* 114, 325-332.

Meseguer, M., Aplin, J.D., Caballero-Campo, P., O'Connor, J.E., Martín, J.C., Remohí, J., Pellicer, A., Simón, C. (2001). Human endometrial mucin MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst. *Biology of Reproduction,* 64, 590-601.

Moore, P.A., Guggenheimer, J., Orchard, T. (2007). Burning mouth syndrome and

peripheral neuropathy in patients with type 1 diabetes mellitus. *Journal of Diabetes Complications,* 21, 397-402.

Mouthaan, J., Sijbrandij, M., Luitse, J.S., Goslings, J.C., Gersons, B.P., Olff, M. (2014). The role of acute cortisol and DHEAS in predicting acute and chronic PTSD symptoms. *Psychoneuroendocrinology,* 45, 179-186.

Nakane, H., Asami, O., Yamada, Y., Ohira, H. (2002). Effect of negative air ions on computer operation, anxiety and salivary chromogranin A-like immunoreactivity. *International Journal of Psychophysiology,* 46, 85-89.

Newhouse, P.A., Dumas, J., Hancur-Bucci, C., Naylor, M., Sites, C.K., Benkelfat, C., Young, S.N. (2008). Estrogen administration negatively alters mood following monoaminergic depletion and psychosocial stress in postmenopausal women. *Neuropsychopharmacology*, 33, 1514-1527.

Nunes, L.A., Mussavira, S., Bindhu, O.S. (2015). Clinical and diagnostic utility of saliva as a non-invasive diagnostic fluid: a systemic review. *Biochemical Medicine,* 25, 177-192.

O'Connor, K.A., Ferrell, R., Brindle, E., Trumble, B., Shofer, J., Holman, D.J.,

Weinstein, M. (2009). Progesterone and ovulation across stages of the transition to menopause. *Menopause,* 16, 1178-1187.

Oberbeck, R., Benschop, R.J., Jacobs, R., Hosch, W., Jetschmann, J.U., Schürmeyer, T.H., Schmidt, R.E., Schedlowski, M. (1998). Endocrine mechanisms of stress-induced DHEA-secretion. *Journal of Endocrinological Investigation,* 21, 148-153.

Parker, L., Eugene, J., Farber, D., Lifrak, E., Lai, M., Juler, G. (1985). Dissociation of adrenal androgen and cortisol levels in acute stress. *Hormone and Metabolic Research,* 17, 209-212.

Paterson, A.J., Lamb, A.B., Clifford, T.J., Lamey, P.J. (1995). Burning mouth syndrome: the relationship between the HAD scale and parafunctional habits. *Journal of Oral Pathology and Medicine,* 24, 289-292.

Patton, L.L., Siegel, M.A., Benoliel, R., De Laat, A. (2007). Management of burning mouth syndrome: systematic review and management recommendations. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics,* 103 Suppl, e1-e13.

Peng, C.H., Xia, Y.C., Wu, Y., Zhou, Z.F., Cheng, P., Xiao, P. (2013) Influencing factors for saliva urea and its application in chronic kidney disease. *Clinical Biochemistry,* 46, 275-277.

Pramanik, R., Osailan, S.M., Challacombe, S.J., Urquhart, D., Proctor, G.B. (2010). Protein and mucin retention on oral mucosal surfaces in dry mouth patients. *European Journal of Oral Sciences,* 118, 245-253.

Priye, A., Bird, S.W., Light, Y.K., Ball, C.S., Negrete, O.A., Meagher, R.J. (2017) A smartphone-based diagnostic platform for rapid detection of Zika, chikungunya, and dengue viruses. *Scientific Reports,* 20, 44778.

Puhakka, A., Forssell, H., Soinila, S., Virtanen, A., Röyttä, M., Laine, M., Tenovuo, O., Teerijoki-Oksa, T., Jääskeläinen, S.K. (2016). Peripheral nervous system involvement in primary burning mouth syndrome- results of a pilot study. *Oral Diseases,* 22, 338-344.

Randolph, J.F. Jr., Zheng, H., Sowers, M.R., Crandall, C., Crawford, S., Gold, E.B., Vuga, M. (2011). Change in follicle-stimulating hormone and estradiol across the menopausal transition: effect of age at the final menstrual period. *The Journal of Clinical Endocrinology & Metabolism,* 96, 746-754

Reinfenstein, E.C., Jr. (1946). Psychogenic or hypothalamic amenorrhea. *Medical Clinics North America,* 30, 1103-1114.

Riggs, B.L., Melton, L.J. 3rd. (1986). Involutional osteoporosis. *The New England Journal of Medicine,* 26, 1676-1686.

Sapolsky, R.M., Krey, L.C., McEwen, B.S. (1986). The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. *Endocrine Review,* 7, 284-301.

Saruta, J., Tsukinoki, K., Sasaguri, K., Ishii, H., Yasuda, M., Osamura, Y.R., Watanabe, Y., Sato, S. (2005). Expression and localization of chromogranin A gene and protein in human submandibular gland. *Cells Tissues Organs,* 180, 237-244.

Saxena, S., Kumar, S. (2015). Saliva in forensic odontology: A comprehensive update. *Journal of Oral and Maxillofacial Pathology,* 19, 263-265.

Scala, A., Checchi, L., Montevecchi, M., Marini, I., Giamberardino, M.A. (2003). Update on burning mouth syndrome: overview and patient management. *Critical Review in Oral Biology & Medicine,* 14, 275-291.

Schiavone, V., Adamo, D., Ventrella, G., Morlino, M., De Notaris, E.B., Ravel, M.G., Kusmann, F., Piantadosi, M., Pollio, A., Fortuna, G., Mignogna, M.D. (2012). Anxiety, depression, and pain in burning mouth syndrome: first chicken or egg? *Headache,* 52, 1019-1025.

Schroten, H., Hanisch, F.G., Plogmann, R., Hacker, J., Uhlenbruck, G., Nobis-Bosch, R., Wahn, V. (1992). Inhibition of adhesion of S-fimbriated Escherichia coli to buccal epithelial cells by human milk fat globule membrane components: a novel aspect of the protective function of mucins in the nonimmunoglobulin fraction. *Infection and Immunity* 60, 2893-2899.

Schwartz, E.B., Granger, D.A. (2004). Transferrin enzyme immunoassay for quantitative monitoring of blood contamination in saliva. *Clinical Chemistry,* 50, 654-656.

Shaywitz, S.E., Shaywitz, B.A., Pugh, K.R., Fulbright, R.K., Skudlarski, P., Mencl, W.E., Constable, R.T., Naftolin, F., Palter, S.F., Marchione, K.E., Katz, L., Shankweiler, D.P., Fletcher, J.M., Lacadie, C., Keltz, M., Gore, J.C. (1999). Effect of estrogen on brain activation patterns in postmenopausal women during working memory tasks. *Journal of American Medical Association,* 7, 1197-1202.

Sherman, B.M., West, J.H., Korenman, S.G. (1976). The menopausal transition:

analysis of LH, FSH, estradiol, and progesterone concentrations during menstrual cycles of older women. *The Journal of Clinical Endocrinology & Metabolism,* 42, 629-636.

Shi, M., Sui, Y.T., Peskind, E.R., Li, G., Hwang, H., Devic, I., Ginghina, C., Edgar, J.S., Pan, C., Goodlett, D.R., Furay, A.R., Gonzalez-Cuyar, L.F., Zhang, J. (2011). Salivary tau species are potential biomarkers of Alzheimer's disease. *Journal of Alzheimer's Disease,* 27, 299-305.

Ship, J.A., Grushka, M., Lipton, J.A., Mott, A.E., Sessle, B.J., Dionne, R.A. (1995) Burning mouth syndrome: an update. *The Journal of American Dental Association* 126: 842-853.

Slomiany, B.L., Murty, V.L., Piotrowski, J., Slimiany, A. (1996). Salivary mucins in oral mucosal defense. *General Pharmacology,* 27, 761-771.

Tabak, L.A. (1995). In defense of the oral cavity: structure, biosynthesis, and function of salivary mucins. *Annual Review of Physiology,* 57, 547-564.

Takiyyuddin, M.A., Brown, M.R., Dinh, T.Q., Cervenka, J.H., Braun, S.D., Parmer, R.J., Kennedy, B., O'Connor, D.T. (1994). Sympatho-adrenal secretion in humans:

factors governing catecholamine and storage vesicle peptide co-release. *Journal of Autonomic Pharmacology,* 14, 187-200.

te Velde, E.R., Pearson, P.L. (2002). The variability of female reproductive ageing. *Human Reproduction Update,* 8, 141-154.

Tsigos, C., Chrousos, G.P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research,* 53, 865-71.

Van der Waal I. (1990) The Burning Mouth Syndrome. Copenhagen: Munksgaard.

Vermes, I., Beishuizen, A. (2001). The hypothalamic-pituitary-adrenal response to critical illness. *Best Practice & Research Clinical Endocrinology & Metabolism,* 15, 495-511.

Vermeulen, A., Deslypere, J.P., Schelfhout, W., Verdonck, L., Rubens, R. (1982). Adrenocortical function in old age: response to acute adrenocorticotropin stimulation. *The Journal of Clinical Endocrinology & Metabolism* 54, 187-191.

Wickström, C., Christersson, C., Davies, J.R., Carlstedt, I. (2000). Macromolecular

organization of saliva: identification of 'insoluble' MUC5B assemblies and non-mucin proteins in the gel phase. *Biochemical Journal,* 15, 421-428.

Wirth, M.M., Meier, E.A., Fredrickson, B.L., Schultheiss, O.C. (2007). Relationship between salivary cortisol and progesterone levels in humans. *Biological Psychology,* 74, 104-107.

Woda, A., Dao, T., Gremeau-Richard, C. (2009). Steroid dysregulation and stomatodynia (burning mouth syndrome). *Journal of Orofacial Pain,* 23, 202-210.

Wood, P. (2009). Salivary steroid assays - research or routine? *Annals of Clinical Biochemistry,* 46, 183-196.

World Health Organization. (1996). Research on the menopause in the 1990s. Technical report Scr 866, Geneva, Switzerland.

Xiao, H., Langerman, A., Zhang, Y., Khalid, O., Hu, S., Cao, C.X., Lingen, M.W., Wong, D.T. (2015). Quantitative proteomic analysis of microdissected oral epithelium for cancer biomarker discovery. *Oral Oncology,* 51, 1011-1019.

Yen, S.S. The biology of menopause. (1977). *The Journal of Reproductive Medicine,* 18, 287-296.

Yilmaz, Z., Renton, T., Yiangou, Y., Zakrzewska, J., Chessell, I.P., Bountra, C., Anand, P. (2007). Burning mouth syndrome as a trigeminal fibre neuropathy: Increased heat and capsaicin receptor TRPV1 in nerve fibres correlates with pain. *Journal of Clinical Neuroscience,* 14, 864-871.

Yolken, R.H., Peterson, J.A., Vonderfecht, S.L., Fouts, E.T., Midthun, K., Newburg, D.S. (1992). Human milk mucin inhibits rotavirus replication and prevents experimental gastroenteritis. *Journal of Clinical Investigation,* 90, 1984-1991.

Table 1. Characteristics of patients with BMS, mean ± S.D., or n (%).

Total participants (n)	30
Age (years)	60.0 ± 5.0
Symptom duration (months)	28.1 ± 39.3
Range	2 - 180
Salivary flow rate (mL/min)	
UWS	0.27 ± 0.17
SWS	1.11 ± 0.48
Type of symptoms	
Burning	28 (93.3)
Aching	18 (60.0)
Itching	6 (20.0)
Stinging	13 (43.3)
Numbness	8 (26.7)
Taste disturbance	18 (60.0)
Dry mouth	20 (66.7)
Sore throat	15 (50.0)
Severity of symptoms (VAS)	
Burning	6.1 ± 2.9
Aching	3.2 ± 3.7
Itching	0.8 ± 2.1
Stinging	2.3 ± 3.2
Numbness	0.9 ± 1.8
Taste disturbance	2.7 ± 3.1
Dry mouth	3.3 ± 2.8
Sore throat	2.6 ± 3.2
Eff-life	4.4 ± 3.0
Area of symptoms	
Tongue	30 (100.0)
Palate	7 (23.3)
Lip	9 (30.0)
Buccal mucosa	2 (6.7)
Gingival/alveolar mucosa	5 (16.7)
Mouth floor	1 (3.3)
Teeth	2 (6.7)
Parafunctional habits	
Pressing the tongue against teeth	8 (26.7)
Diurnal clenching	6 (20.0)
Nocturnal clenching and/or bruxism	3 (10.0)
Tongue and/or cheek biting	3 (10.0)

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

Table 2. Salivary levels of cortisol, DHEA, cortisol/DHEA, 17ß-estradiol, progesterone, CgA, and blood contamination in patients with BMS.

n = 30	Mean ± S.D.
Cortisol (μg/dL)	
UWS	0.28 ± 0.25
SWS	0.24 ± 0.16
DHEA (pg/mL)	
UWS†	65.3 ± 45.0
SWS	54.3 ± 35.9
Cortisol/DHEA	
UWS†	79.4 ± 87.2
SWS	73.6 ± 74.0
17ß-Estradiol (pg/mL)	
UWS	1.01 ± 0.56
SWS	1.21 ± 0.45
Progesterone (pg/mL)	
UWS	40.2 ± 41.6
SWS	24.5 ± 18.4
CgA (pmol/mL)	
UWS†	62.3 ± 37.1
SWS	40.0 ± 18.0
Blood (mg/dL)	
UWS	0.83 ± 0.45
SWS	0.36 ± 0.20

BMS, burning mouth syndrome; UWS, unstimulated whole saliva; SWS, stimulated whole saliva; DHEA, dehydroepiandrosterone; CgA, chromogranin A; Blood, blood contamination

† In one UWS sample, assays of DHEA and CgA could not be performed due to a shortage of sample (n = 29).

Table 3. Correlation coefficients between the results of salivary analyses and clinical symptoms.

n = 30	Symptom duration	Burning (VAS)	Eff-life (VAS)
MUC1	-.215	.055	.093
Flow rate			
UWS	.141	.177	.120
SWS	-.033	.209	-.019
Cortisol			
UWS	-.204	-.391*	-.357
SWS	-.239	-.202	-.294
DHEA			
UWS†	-.056	.316	.068
SWS	-.210	.226	-.010
Cortisol/DHEA			
UWS†	-.140	-.517**	-.246
SWS	.103	-.401*	-.139
17β-Estradiol			
UWS	.626**	.184	.165
SWS	.250	.277	.397*
Progesterone			
UWS	-.166	.161	-.016
SWS	-.167	.065	.072
CgA			
UWS†	.094	-.265	-.185
SWS	.020	-.327	-.273
Blood			
UWS	-.261	-.405*	-.149
SWS	-.014	-.222	.229

VAS, visual analog scale; UWS, unstimulated whole saliva; SWS, stimulated whole saliva; DHEA, dehydroepiandrosterone; CgA, chromogranin A; Blood,

blood contamination; Eff-life, the effect of oral complaints on daily life

† In one UWS sample, assays of DHEA and CgA could not be performed due to a shortage of sample (n = 29).

$^{*}P < 0.05$, $^{**}P < 0.01$ by Pearson's correlation analysis

Table 4. Correlations between levels of oral mucosal MUC1 expression, salivary flow rate, female gonadal hormones, and stress markers in UWS.

n = 30	Flow rate	Cortisol	DHEA†	Cortisol /DHEA†	17β-Estradiol	Progest-erone	CgA†	Blood
MUC1	.002	.186	.037	.009	.065	.550**	.305	.304
Flow rate		-.041	.242	-.225	.045	.074	-.203	-.199
Cortisol			.332	.182	.026	.428*	.125	.248
DHEA†				-.633**	.346	.510**	.071	.045
Cortisol /DHEA†					-.278	-.229	.010	.306
17β-Estradiol						.418*	-.104	-.299
Progest-erone							.058	.080
CgA†								.571**

UWS, unstimulated whole saliva; DHEA, dehydroepiandrosterone; CgA, chromogranin A; Blood, blood contamination

† In one UWS sample, assays of DHEA and CgA could not be performed due to a shortage of sample (n = 29).

*$P < 0.05$, **$P < 0.01$ by Pearson's correlation analysis

Table 5. Correlations between levels of oral mucosal MUC1 expression, salivary flow rate, female gonadal hormones, and stress markers in SWS.

n = 30	Flow rate	Cortisol	DHEA	Cortisol /DHEA	17β-Estradiol	Proges-terone	CgA	Blood
MUC1	-.052	.106	-.127	0.214	-.117	.414*	.142	.038
Flow rate		.258	.173	-.173	-.245	.181	-.439*	-.352
Cortisol			.477**	-.022	-.074	.409*	.008	.114
DHEA				-.626**	.363*	.538**	-.024	.231
Cortisol /DHEA					-.214	-.199	.155	-.122
17β-Estradiol						.314	-.003	.196
Proges-terone							-.142	.030
CgA								.440*

SWS, stimulated whole saliva; DHEA, dehydroepiandrosterone; CgA, chromogranin A; Blood, blood contamination

*$P < 0.05$, **$P < 0.01$ by Pearson's correlation analysis

Supplementary Table S1. Correlation coefficients between symptom duration, severities of symptoms and Eff-life, and results from psychological evaluation.

	SOM	O-C	I-S	DEP	ANX	HOS	PHOB	PAR	PSY	GSI	PSDI	PST
Symptom	.052	.363*	.139	.214	-.028	.312	-.052	.214	.031	.155	.248	.101
Burning	.221	.231	.120	.202	.096	.317	.076	.185	.269	.264	.252	.227
Aching	.162	.167	.150	.207	.205	.380*	.343	.104	.286	.254	.219	.182
Itching	.121	.071	-.066	.083	-.072	.190	.014	.010	.014	.100	.166	.091
Stinging	.199	.207	.342	.325	.322	.360	.275	.326	.198	.348	.108	.337
Numbness	.195	.336	.280	.169	.250	.272	.751**	.304	.308	.361*	.124	.443*
Taste disturbance	.305	.216	.265	.492**	.418*	.238	.407*	.152	.383*	.412*	.297	.308
Dry mouth	.348	.266	.080	.057	.125	.143	.304	.198	.149	.275	.160	.307
Sore throat	.265	-.069	-.241	-.170	-.157	.003	.176	-.197	.010	.025	.237	-.049
Eff-life	.152	.223	.084	.219	.091	.377*	.350	.121	.288	.261	.323	.173

SOM, somatization; O-C, obsessive-compulsive; I-S, interpersonal sensitivity; DEP, depression; ANX, anxiety; HOS, hostility; PHOB, phobic anxiety; PAR, paranoid ideation; PSY, psychoticism; GSI, global severity index; PSDI, positive symptom distress index; PST, positive symptom total; Eff-life, the effect of oral complaints on daily life

*$P < 0.05$, **$P < 0.01$ by Pearson's correlation analysis

Supplementary Table S2. Correlation coefficients between level of oral mucosal MUC1 expression, salivary flow rates, levels of female gonadal hormones and stress markers, and results from psychological evaluation.

	SOM	O-C	I-S	DEP	ANX	HOS	PHOB	PAR	PSY	GSI	PSDI	PST
MUC1	-.088	-.219	-.217	-.361	-.348	-.183	-.336	-.144	-.296	-.267	-.139	-.290
Flow rate												
UWS	-.167	-.245	-.186	-.283	-.234	.071	-.135	.138	-.152	-.201	-.235	-.111
SWS	-.077	-.164	-.086	-.227	.030	.024	-.018	.037	-.088	-.084	-.214	.001
Cortisol												
UWS	-.085	-.301	-.033	-.284	-.076	-.234	-.182	-.129	-.234	-.230	-.123	-.275
SWS	-.152	-.229	-.095	-.196	.009	-.228	-.216	-.125	-.253	-.201	-.051	-.225
DHEA												
UWS[†]	-.056	-.131	-.182	-.011	.203	-.158	-.191	-.216	-.016	-.062	.064	-.145
SWS	.057	-.113	-.032	.151	.329	-.125	-.148	-.161	.187	.056	.014	.054
Cortisol/DHEA												
UWS[†]	-.084	-.090	.018	-.079	-.249	.027	.012	-.143	-.209	-.128	-.164	-.105

SWS	-.208	.019	-.157	-.208	-.350	.015	-.121	-.176	-.378*	-.242	-.096	-.274
17β-Estradiol												
UWS	.054	.412*	.116	.186	.077	.160	-.182	.027	-.007	.124	.195	.032
SWS	.278	.503**	.251	.420*	.341	.304	.134	.166	.461*	.397*	.270	.399*
Progesterone												
UWS	.178	.053	-.023	-.149	-.032	-.074	-.230	-.019	-.100	.020	.027	-.005
SWS	.170	.026	.009	-.079	.087	-.052	-.163	-.066	-.016	.029	-.064	.043
CgA												
UWS†	-.010	-.240	-.298	-.118	.122	-.217	-.192	-.238	-.108	-.145	.016	-.206
SWS	-.230	-.194	-.386*	-.106	-.083	-.475**	-.357	-.367*	-.181	-.268	.104	-.337
Blood												
UWS	-.133	-.433*	-.310	-.107	.009	-.308	-.130	-.350	-.287	-.223	-.154	-.260
SWS	-.052	-.193	-.035	.193	.212	-.082	.107	-.095	.087	.030	.120	.000

UWS, unstimulated whole saliva; SWS, stimulated whole saliva; DHEA, dehydroepiandrosterone; CgA, chromogranin A; Blood, blood contamination; SOM, somatization; O-C, obsessive-compulsive; I-S interpersonal sensitivity; DEP, depression; ANX, anxiety; HOS, hostility; PHOB, phobic anxiety; PAR, paranoid ideation; PSY, psychoticism; GSI, global severity index; PSDI, positive symptom distress index; PST, positive symptom total

† In one UWS sample, assays of DHEA and CgA could not be performed due to a shortage of sample (n = 29).

*$P < 0.05$, **$P < 0.01$ by Pearson's correlation analysis

국문 초록

Relationships between Oral MUC1 Expression and Salivary Hormones in Burning Mouth Syndrome

구강작열감증후군 환자의 구강점막 상피세포 MUC1의 발현과 타액 스트레스 호르몬 및 여성 호르몬 농도의 상관관계

강 정 현

서울대학교 대학원

치의과학과, 구강내과·진단학 전공

(지도교수: 고 홍 섭)

구강작열감증후군은 복잡하고 다양한 병인으로 인하여 발생하는 질환으로 신경계-정신계-내분비계 조절 기전 이상 및 구강점막의 완전성 저하가 주요한 병인 중 하나로 생각되고 있다. 이번 연구는 구강작열감증후군 환자에서 구강상피 MUC1의 발현량과 타액 내 여성 호르몬과 스트레스 표지자의 농도 및 임상적 증상과의 상관관계를 비교하고자 시행되었다.

본 연구에서는 폐경 이후의 여성 구강작열감증후군 환자 30명

(평균 60.0 ± 5.0세)을 대상으로 임상검사 및 Symptom Checklist-90-Revised를 활용한 심리학적 평가를 시행하였으며 구강점막 상피세포 MUC1의 상대적 발현량을 측정하였다. 그리고 비자극성 타액과 자극성 타액 검체를 이용하여 cortisol, dehydroepiandrosterone, 17β-estradiol, progesterone, chromogranin A의 농도와 혈액오염 정도를 측정하였다.

연구 결과, 타액 내 progesterone의 농도는 구강점막 상피세포 MUC1의 발현 정도, 타액 내 cortisol 농도 및 DHEA 농도와 유의미한 양의 상관관계를 보였다. 타액 내 17β-estradiol 농도는 구강 증상의 기간, 구강 증상으로 인한 일상 생활의 지장 정도 및 심리학적 평가 결과와 유의미한 양의 상관관계를 보였다. 비자극성 타액 내의 cortisol 농도와 비자극성 및 자극성 타액 내에서의 cortisol/DHEA 비율은 구강작열감의 심각도와 유의미한 음의 상관관계를 나타내었다. 이와 함께 미각이상으로 인한 불편감의 정도는 심리학적 평가 결과와 유의미한 양의 상관관계를 보였다.

결론적으로 심리내분비학적 상호조절 기전 이상은 폐경기 구강작열감증후군 환자의 구강점막 상피세포 MUC1의 발현 및 구강작열감의 정도에 영향을 미침을 알 수 있었다.

주요어: MUC1, 스트레스, 성호르몬, 타액, 구강작열감증후군

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